ADVANCES IN FOOD SCIENCE AND NUTRITION

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PREFACE

It is the first edition of **SCIENCE AND EDUCATION DEVELOPMENT INSTITUTE** for food world. It gives me great pleasure in bringing out book entitled **"ADVANCES IN FOOD SCIENCE AND NUTRITION"** for the student of Food Technology, Food Nutrition and all those aspirants who desire to brighten their career in the field of food technology.

Our goal is to provide readers with introductory foundation to budding food professionals. I was also well aware that the book is widely used as a basic reference outside the academic environment. I have attempted to take utmost care to cover the particular topic with latest research updates. In this concern I had compiled all the newer areas of food science and nutrition with the older existing knowledge. I also hope to provide insight into the scope of food science for people considering food science as a career. The book chapters introduces and complex interrelationship among food functional properties, processing, distribution and storage. Every chapter has been extensively appropriate and justified with suitable diagrams and tables. Multicolor picture have been also added to enhance the content value and to give the students an idea of what he will be dealing in reality, and to bridge the gap between theory and practice. I am indebted to Mr. Abulude, F.O**.** President/CEO, Science and Education Development Institute (SEDInst), AKURE, ONDO STATE, NIGERIA for giving an opportunity for this book project, also Mr. Adeyemi Adewale for editing the book. Relevant information on food sector has also been given. Moreover at the end of text Appendices have been given through which the readers can be benefitted. We are confident that this edition will again receive your overwhelming response.

Chapter 1
It contains an introduction to general aspects which covers the chemical properties for food, nutritional properties of proteins and functional properties of foods. It covers some factors that affect the functionality of protein. The paper discusses the importance of these functions when preparing food formulations.

Chapter 2
This chapter is mainly focused on pasting properties of starch. It includes determining its applications in food processing and other industries. Details of the RVA as tool for obtaining information related to apparent viscosity. Pasting properties have been used to predict the end-use quality of various products.

Chapter 3
Chapter includes the details of various types of phenolic compounds, extraction, significance and antioxidative action of phenolic compounds. This topic based on natural phenolic compounds with antioxidant activity such as grape seed extract, rosemary extract, tea, catechin and tannins etc. The main purpose of using an antioxidant as a food additive is to maintain the quality of that food and to extend its shelf life rather than improving the quality of the food.

Chapter 4
The chapter includes basic information on edible film-coating formulation, Specific requirements, properties, methods of application to food surfaces. Topic will also give information about the advantages and disadvantages of edible film.

Chapter 5
The chapter has more focus on carrot production, cultivation, harvesting and post harvest management. It gives information about nutritional significance and changes occur during storage.

Chapter 6
The chapter gives more information about the application of integrated nutrigenomics approach in nutritional sciences. It also emphasized for accelerated implementation of mechanistic knowledge in food design. It gives an application and modification approaches of proteomic to analyze the complexity of food protein modification in the area of general food science and quality assurance.

Chapter 7
The topic includes the details of coping difficult conditions in the Sahel by mobile pastoralist communities in the Lake Chad area. It gives the basic recommendations for Food Insecurity in Africa and particularly in Sahel area.

Chapter 8
The topic concludes spirulina share his remarkable qualities, ease of culture and safety can be an effective and lasting solution to the problems of malnutrition. It gives the details of the pilot project for the development of industry of "Dihe".

Chapter 9
The chapter includes raw materials, process, packaging, PFA specification, types, and health benefits of Pickles. It gives an idea to overcome protein based malnutrition by providing nutrient security by means of pickle.

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Food Functional Properties: A Review

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ABSTRACT

This chapter covers the chemical properties for food, nutritional properties of proteins and functional properties of foods. It covers some factors that affect the functionality of protein. The paper discusses the importance of these functions when preparing food formulations.

INTRODUCTION

Functional properties have been defined as those physical and chemical properties that influence the behaviour of proteins in food systems during processing, storage, cooking and consumption (LabReports. Info, (2011), Alfaro *et al*., (2004). Food products are generally complex systems with several components, such as water, .protein, lipid, carbohydrates, minerals and a number of minor components. These components can interact in various ways to affect the functional and nutritional properties of food products.

Proteins are important in food processing and food product development not only as because of their nutritional properties, but also because they are responsible for many functional characteristics that influence consumer acceptance of food. For efficient utilization and consumer acceptance of a protein product, studies on the functional properties are important (Alli *et al*., 2010).

The aim of this paper is to review the various functional properties that apply to food industry.

(1) The Chemical Properties for Food

This can be considered under the following sub-headings; The water content which is also known as moisture content, ash content, fat content, carbohydrate content and also crude fibre content.

a. Water/Moisture Content

Moisture content is the amount of water present in a food substance. The contents and the physical states of water in food influence the physical, chemical quality and functional properties of food components.

The nutritive value of the food can be determined using the knowledge of moisture content. In order to determine the moisture content the following factors must be considered. The nature of the food, the relative amount of water that is present and the form in which water is present.

The moisture content enables us to know the dry matter content and predicts the appropriate types of preservation technique necessary for a kind of food substance. It also enables us to know the actual amounts of water present in food and the amount to be added during processing.

Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of different reasons:

- *Legal and Labeling Requirements.* There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.
- *Economic.* The cost of many foods depends on the amount of water they contain - water is an inexpensive ingredient, and manufacturers often try to incorporate as much as possible in a food, without exceeding some maximum legal requirement.
- *Microbial Stability.* The propensity of microorganisms to grow in foods depends on their water content. For this reason many foods are dried below some critical moisture content.
- *Food Quality.* The texture, taste, appearance and stability of foods depend on the amount of water they contain.
- *Food Processing Operations.* Knowledge of the moisture content is often necessary to predict the behavior of foods during processing, *e.g.* mixing, drying, flow through a pipe or packaging (LabReports. Info, 2011).

b. Protein Content

Proteins are complex nitrogenous organic compounds occurring naturally in all living matter and forming an essential an part of animal food requirements (Alfaro *et al*., (2004). They are biopolymers of certain L-&-amino or amino acids, the monomers are arranged in specific sequence and joined together primarily in peptide linkage (simple protein). Some may either be covalently or non-covalently combined to a non-protein moiety such as carbohydrates, nucleic acids or lipids conjugated protein. Many proteins have been found to contain almost the same kinds of amino-acids, thereby differing from one another only with respect to the number and sequence of their constituent amino-acids, and with respect to their conformation. Hence, the physical and chemical properties of various proteins vary only slightly. Proteins can be classified on the basis of their solubility. Alli *et al*., (2010) recorded that industrial applications of proteins are limited, because proteins are generally unstable with heating, organic solvents and proteolytic degradation. Modification of proteins has been investigated to improve their physical functionality i.e gelation, viscosity, emulsification and foaming.

Protein and amino acids are essential for healthy replacement of tissues, hormones, antibodies, pigments and synthesis of enzymes (Adeyeye and Omolayo, 2011).

c. Ash Content

The ash content of a food-stuff is the organic residue remaining after the organic matter has been burnt away. The ash figure can be regarded as a general measure of quality and is a useful criterion in identifying the food. The ash obtained may be exactly the same composition as the mineral matter present in the original food there is no loss due to volatilization of some interaction between constituent carbohydrates.

d. Carbohydrate Content

Carbon in hydrate water molecule is called carbohydrates, which have the molecular formula of (CH_2O). They are called carbohydrates because.

They contain hydrogen and oxygen in the same proportion as in water. They are made up of mainly sugar and starch. Carbohydrates are the largest constituents of animal foods and produce energy where oxidized in the body with carbon dioxide and water as the end products.

$$C_6H_{12}O_{6\ (s)} + 6O_2 \longrightarrow 6H_2O\ (1) + 6CO_{2\ (g)} + \text{Energy}.$$

e. Minerals

Minerals are nutrients that exist in the body and in food both in organic and inorganic combinations. There are seventeen minerals essential in human nutrition, just only 5-5% of human body weight is mineral matter (Lin *et al.,* 1975).

Minerals are of two types, the macro-elements and micro-elements. The macro elements are the mineral elements required in the body in appreciable amount and they include Cl, Na, K, S, Mg, Ca, P. The other one which is the micro elements are the mineral elements required in the body in minute quantity and they include Fe, Co, I, Mn, and Zn.

(2) Functional Properties

Functional properties are the intrinsic physico-chemical characteristics of the food substance, which may meet the behaviour of food system during processing and storage (Oshodi and Ekperigin (1989). Functional and physicochemical properties of food proteins are important in food processing and food formulation, since they contribute to obtain consumers desired characteristic and quality (Furlan *et al*., 2011). Properties such as solubility, swelling and viscosity, texture, water and fat binding, emulsion, foam and gel characteristics are of general interest because they are among the most important quality determinants in food formulation (Jayasena *et al*., 2010).

a. Gelation Capacity

This is the gel formation of the protein in suspension. It is defined as the aggregation of denatured protein molecules. Gelation involves the formation of a continuous network which exhibits certain degree of orders (Abulude, 2004). The gelation capacity of legume protein was attributed to the gelation fraction suggesting that these properties would make the protein very useful in food systems such as pudding and sources which require thickening and gelling (Moure *et al*., 2006). Food processing and development of new products require ingredients such as the gelling agents, which build up a structural matrix that supplies food's desirable texture (Alleoni and Cladia, 2006). According to Gbogouri *et al*., (2004), the nature and properties of gels are influenced by several factors, such as protein concentration, solution pH, nature, and concentration of electrolyte.

In one of the works of Alleoni and Cladia, (2006), it was emphasized that intermolecular disulphide linkages increases the stability of the gel matrix. According to Akin-Osanaiye *et al*., (2009), gelation is often an aggregation of denatured molecules and contrary to

coagulation where the aggregation is random, gelation involves the formation of a continuous network which exhibits a certain degree of order.

b. Foaming Capacity

Foam is a material made up of gas bubbles separated from one another by films or liquids. The bubbles are spherical when the liquid films separating them are thick, which is approximately 1mm. they have a polyhedral shape when the liquid films between them are thin. Pure liquid does not foam.

The foaming capacity of a particular food depends on the amount of protein solubilized Oshodi and Ekperigin (1989). The foam capacity of protein is important in production of confectionery and bakery products. Foaming formation is governed by three factors: including transportation, penetration and reorganization of the molecule at the air –water interface. Therefore, to exhibit good foaming, a protein must be capable of migrating at the air-water interface, unfolding and rearranging at the interface (Ogunwolu *et al.,* 2009). According to Saetae and Suntornsuk (2011), factors affecting foam capacity of proteins are the type of protein, degree of denaturation, pH, temperature and whipping methods. Foam can be produced by whipping air into liquid quickly as much as possible. Low foaming capacities could be due to inadequate electrostatic repulsions, lesser solubility and excessive protein-protein interactions (Kinsella (1979), Butt and Batool (2010).

From the research works carried out by Moure *et al*., (2006); Mepba *et al*., (2008); Akin-Osanaiye *et al*., (2009); Ekpo and Ugbenyen (2011); Martin, (2010); and Eltayeb *et al*., (2010), it was observed that the foaming capacity of their samples increased as the weight and pH increased.

c. Water Absorption Capacity

This is water-binding capacity and it denotes the water retained (bound and entrapped by the protein after centrifugation).

This measures the extent to which dietary protein in food will retain water. This is an important property which enables bakers to add more or less water to the dough, this improves its characteristics and maintains the freshness of bread and increases the palatability of baked food.

d. Oil Absorption Capacity

This is one of the surface-active properties of protein. The capacity of protein to absorb at interface and form cohesive molecules is critical in emulsions and foams. The oil absorption may merely be another aspect of emulsification.

The amount of oil bounds is markedly affected by the method used, the protein content, the charge area, the surface area, hydrophobicity and liquidity of the oil. The mechanism of fat absorption is attributed mainly to the physical entrapment of oil and the binding of fat to the polar chain of protein (Adeleke and Odedeji, 2010).

e. Fat Emulsion Capacity

Fat emulsion capacity is the extent to which the dietary protein will mould dietary oil into fine particles. It directly measures the extent to which the dietary protein will mix oil. This is important in the sense that proteins in foods are always emulsified during digestion.

Emulsion of fat and water are thermodynamically unstable because of the high free energy contributed by the interfacial tension. Emulsion capacity measurements involve two-step processes at the end point, the large globular structure breaks down at first releasing oil droplets which then coalescence.

f. Solubility

The ability of a protein to dissolve in water is known as solubility and this facilitates the incorporation of additives into food. Protein solubility measured the solubility of the food protein in solution or in suspension. The solubility property is pH dependent and is important in that it gives the least solubility pH value (Isoeletric point) of a given food sample and indicates the soluble protein at physiological pH.

Under given condition, like pH, temperature and ionic strength, protein solubility is the manifestation of the equilibrium between protein-solvent and protein interactions.

Solubility measurement gives valuable information on a protein ingredient. The pH dependency reflects the complexity of a protein system. A solubility profile with a low solubility over a broad pH range is a sign of serve denaturation and insolubilization. High solubility data are sometimes obtained from completely denatured protein. The solubility of protein decreases during storage of flour (Pour-El, 1979).

In a research work carried out by Gbogouri *et al*., (2004) on fish, they remarked that high solubility of fish protein hydrolysate over a wide range of pH is substantially useful characteristic for many food applications. Furthermore, it influences the other functional properties such as emulsifying and foaming properties. However, a very high degree of hydrolysis can have enormously negative effects on the functional properties (Altschul and Wilcke, 1985).

(3) Functional Properties of Protein

Proteins have no parallel in their structural and textural versatilities. Although nature has designed proteins to perform specific roles within the body system, they can display multifunctional properties by appropriate manipulation and processing treatments in different food systems.

Vegetable and oil sees constitute an enormous source of protein for human consumption (Altschul and Wilcke, 1985) (Table 1). However, to be exploited successfully, these proteins must be presented to the consumers in forms that are attractive and process the flavour, texture and quality desired by the consumers. The properties of proteins that determine their uses in foods are collectively called functional properties (Altschul and Wilcke, 1985).

Functional properties denote those physicochemical properties of food proteins that determine their behaviour in foods during processing, storage, preparation and

consumption. It depends on such intrinsic physicochemical characteristics of protein as amino acid composition and sequence, molecular weight, conformation and charge density facilitates interaction with other food components such as water, ions, lipids, carbohydrates, vitamins, color and flavour constituents, depending upon the environment pH, ionic strength and temperature during preparation, processing and storage (Altschul and Wilcke, 1985). The type of functional property required in a protein or a protein mix varies with the particular food system in question. Thus for example, water binding, solubility, swelling, viscosity, gelation, and surface activity are important properties determining usefulness and final product quality in meat system (Adeyeye *et al*., 2005).

Table 1: Proximate composition (%) of the major oilseeds of commercial importance.

Seed	Oil	Protein	Carbohydrate	Moisture
Soyabean `	24	40	29	7
Peanut	49	24	18	9
Dehulled cotton seed	33	38	22	7
Sunflower seed	40	30	25	5
Rape seed	35	28	35	2

Source: Altschul and Wilcke, (1985).

Although the importance of functional properties (Table 2) has been recognized by commodity specialists (i.e Dairy and Cereal scientists) for many years, the general recognition of their importance in food processing has been accelerated by their increased emphasis on food manufacturing and formulation with functional ingredients. Universal attempts to use less expensive oil seed proteins to extent traditional foods (texture by soy bits), to develop new functional ingredients (gelling, foaming, emulsifying and acid-soluble proteins), to fabricate new food analogues and to stimulate traditional foods (milk, meats and whipped topping) have focused attention on the critical role of functional properties in determining the application of oilseed proteins in foods Altschul and Wilcke, (1985).

Table 2: Functional properties of oilseed proteins of importance in food application.

General property	Specific Functional Attributes
Organoleptic	Colour, flavor, odour
Kinesthetic	Texture, mouth feel, smoothness, grittiness, turbidity, chewness
Hydration	Wettability, water absorption, water holding capacity, Swelling, solubility, thickening, gelling, syneresis.
Surface	Emulsification, foaming, (aeration, whipping) protein -lipid binding, flavour binding
Structural and Rheological	Viscosity, elasticity, adhesiveness, cohesiveness, Stickness, dough formation, aggregation, gelation, network formation, extrudability, texturizability, fibre formation.
Others	Compatibility with other food components, enzymatic activities, anti oxidant properties

Source: Kinsella, (1979).

(4) Nutritional Properties of Proteins

Many methods of classifying the functional properties of protein products have been proposed (Pour-El, A (1979), Alleoni and Cladia (2006), Adeyeye *et al*., (2005). They are based on the preferences and intentions of the researchers.

Aims of Utilization: These are the main divisions of functionality and include sense affecting manipulation and industrial properties. Enzymic characteristics are also functional properties of some protein products.

a. Material Tested

Classified as to whether the material is a good product (including feeds) or a food ingredient. The former functionality affects its utilization by the consumer while the latter is more important to the processor.

b. Test Procedure

This divides the functional properties into a model system (or model Test-an evaluation of one or more functional properties that does not mimic completely the steps and ingredient of an actual food preparation) or utility system (or utility test, or food-Test-an evaluation of a functional property that mimics food preparation in all its particulars).

c. Evaluation Procedure

Functionality is evaluated either by subjective, semi objective, or objective methods. The subjective methods utilize exclusively the five senses as sensors and the brain as the value assigner. Objective methods utilize completely artificial sensors and instruments as the value assigner.

(5) Physico-Chemical Properties

Assigns to each of these molecular properties some functional characteristics that are influenced by it as follows:

a. Hydrophillic

Properties depend on the affinity of the protein for water and other polar solvents, including solubility, water uptake and wettability. Due to their hydrophilic (water - loving) and hydrophobic (oil – loving) structures, proteins are amongst the most effective emulsifiers commonly applied in the food industry. The outer hydrophilic hydrocarbons terminals can interact with the surrounding aqueous phase while the lipophilic interphases interact with the surrounding oil phase. This results in the reduction of the interphase tension between molecules of one immiscible liquid and another immiscible liquid (for example, oil and water) (Jayasena *et al*., 2010).

b. Interphasic

Properties depend on the ability of the protein molecule to form separation on junction films between two immiscible media, including emulsification, fat uptake, foaming, absorption and conservation.

c. Intermolecular

Properties utilizing the ability of proteins to form junctions of its own molecules to themselves or to other components including viscosity, thickening, gelation, film formation, foaming fiber formation, adhesion, cohesion, sickness, hardness, complex formation, spreading, elasticity and plasticity.

d. Organoleptic

Properties of proteins manifested through the sense organs including odour (nose), colour (eye), flavour (taste organs), brittleness (ear) and so forth.

(6) Other Effects

Special chemical and physical properties that pertain to specific proteins or example are incompatibilities.

Protein Properties and Functionality

The factors influencing the functional properties of protein products are the innate characteristics (physcio-chemical) of proteins and processing and modification steps that alter them. Physico-chemical properties include:

a. Amino Acid Composition

The percentage and distribution of the amino acid has great influence on hydrophobicity. It also influences the rate of processing and modifying alternations in its nature (Pour-El, 1979). It is well documented that most leguminous plant seeds are rich in nutrients such as digestible protein with a good array of amino acids and minerals (Fagbemi *et al*., (2004), Aletor and Ojelabi (2007).

b. Size

The larger molecule units in the materials the more insoluble it becomes. It also influences the ease of disintegration and rearrangement necessary during modification (Pour-El, 1979).

c. Conformation

The conformation characteristics are involved in functional properties through hydrophilicity, gelation and film forming among others.

d. Denaturation

This change in conformation has always been considered the bane of protein processing prior to final form formation. At that step, controlled denaturation often is practiced (Pour-El, 1979).

e. Bonds and Forces

These properties are the mediators affecting the changes in size and conformation. Van der Waal forces, ionic bonds, hydrogen bonds, covalent bonds and hydrophobic bonds all play part in the original protein structure as well as in the modification leading to altered functionality. Adequate correlations of these with functional properties are the subjects of "Functional Evaluations" (Pour-El, 1979).

(7) Factors Affecting Functional Properties of Proteins

There are seven major process steps that usually bear some influence on the functionality of the protein products as follows:

a. Protein Source and Variety

By choosing a particular protein, one many increase a particular functionality; for example, increased sulphur amino acid concentration will enhance gelation of protein ingredients (Pour-El, 1979).

b. Temperature

The denaturation effect of temperature will have a very significant effect on functionality. One must also mention the possibility of alterations through annealing (the keeping of a material at a constant temperature for a time period) on certain functionalities such as water absorption.

c. Drying

The methods of drying influence functionality through the temperature used. Modifications of porosity and particle size are also frequently observed during this treatment (Pour-El, 1979).

d. Ionic Theory

The pH history of the protein sample influences its state of naturation and its conformation. Also, the presence of particular ions will sometimes drastically influence its functionality through formation of complexes with diverse properties.

e. Impurities

The presence of the components such as fats, soluble carbohydrates, sugar, etc. has a great effect on the functionality of the protein product. Complexing reactions may occur that severely alter the properties (for example, grittiness resulting from insoluble components).

f. Storage

Storage affects the stability of a protein product to varying degree. Even cold from storage, while apparently not detrimental to the state of naturation, may sometimes result in other chemical reactions, not all of which are beneficial. The moisture content during storage is a well known factor affecting product characteristics (Pour-El, 1979).

(8) FOODS AND FUNCTIONAL PROPERTIES

Table 3 is the list of foods and the functional properties needed for their success.

Table 3: Types of foods and their related functionalities.

Type	Functionality
Beverage	Solubility, grittness, colour.
Baked goods	Emulsification, complex formulation, Foaming, visco elastic properties, matrix, and film formation, gelation, hardness, absorption.
During substitutes	Gelation, coagulations, foaming, fat holding capacity.
Egg substitutes	Foaming and gelation.
Meat extenders	Liquid holding capacity, hardness, chewiness, cohesion, adhesion.
Soups and gravies	Viscosity, emulsification, water absorption.
Toppings	Foaming, emulsification.
Whipped deserts	Foaming gelation, emulsification.

Source: Kinsella (1976).

Testing a Protein Product for Functional Properties

Chemical analysis for proximate composition are essential to adequately carry out future functional testing, the percentage of protein and other components will invariably supply clues as to the expected functional properties. Physical analysis for size of particle, appearance and density will also aid in the search for good functionality (Pour-El, 1979).

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2

Pasting Properties of Rice

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INTRODUCTION

Rice flour and starch are important ingredients in both traditional and novel foods. In recent years, rice, especially rice flour, because of its unique functional properties, is being used in novel foods such as tortillas, beverages, processed meats, puddings, salad dressing, and gluten-free breads (Kadan, R.S., & Ziegler G.M. 1989; McCue, 1997; Kadan, R.S., Robinson, M.G., Thibodeux, D.P., & Pepperman, A.B., Jr.2001). In Western countries, rice is widely used to manufacture products such as puddings, infant foods, puffed grains and breakfast cereals. In order to control better production processes, it is necessary to understand the properties of rice flour and starch.

Pasting properties

Granules consist of starch molecules. These starch molecules are arranged radically and form a series of concentric layers with alternate amorphous and semi-crystalline regions. Each starch molecule is a large polymer consisting of glucose units. There are two distinct polymer types: amylose and amylopectin. Amylose is a relatively small polymer with a linear structure, whereas amylopectin is a very large polymer that exhibits substantial branching. Waxy starches contain very little amylose. Other starches generally contain 15–30% amylose, depending on the starch type. During a standard starch analysis, the starch is heated in an aqueous environment. The starch granule imbibes water and swells, the internal crystalline structure melts (gelatinization), the granule itself breaks down and a continuous gel forms. The relatively soluble amylose leaches out into solution, followed at a slower rate in some cases by the amylopectin. Owing to the mechanical shear applied to the sample, these polymers then tend to align themselves. These combined processes that follow gelatinization are known as pasting. The viscosity changes produced by heating and cooling starch in water generally provide a similar characteristic pasting curve.

Pasting parameters

- The **pasting temperature** provides an indication of the minimum temperature required to cook a given sample, which can have implications for the stability of other components in a formula and also indicate energy costs. Higher amylose types generally show higher pasting temperatures on RVA (Suzuki 1979, Perez, C.M., Villareal, C.P., Juliano, B.O., & Biliaderis, C.G. 1993).
- The **peak viscosity** occurs at the equilibrium point between swelling and polymer leaching (which cause viscosity to increase) and rupture and polymer alignment (which cause viscosity to decrease). Peak viscosity indicates the water-binding capacity of the starch or mixture. It is often correlated with final product quality, and

also provides an indication of the viscous load likely to be encountered by a mixing cooker.

- The temperature and time corresponding to the peak viscosity are referred to as the **peak temperature and peak time**.
- During the hold period of a test, the sample is subjected to a period of constant high temperature (typically 95°C) and mechanical shear stress. The granules undergo further disruption while the amylose molecules continue to leach out into solution and undergo alignment. This period is usually accompanied by a reduction in viscosity, which eventually reaches a minimum value. This viscosity is known either as the **holding strength, hot paste viscosity or trough**. The rate and extent of reduction from the peak viscosity depend on the temperature, the degree of mixing or shear stress applied to the mixture, and the nature of the material itself. The ability of a sample to withstand this heating and shear stress is an important factor for many processes. Cross-linked starches, for example, are resistant to such breakdown.
- As the mixture subsequently cools, re-association between starch molecules, especially amylose, occurs to a greater or lesser degree. Given a sufficient concentration of starch, this usually causes the formation of a gel. The viscosity will normally increase and stabilize at a final viscosity. **Final viscosity** is the most commonly used parameter to define a particular sample's quality, as it indicates the ability of the material to form a viscous paste or gel after cooking and cooling.
- The phase of the pasting curve between the trough and final viscosity is commonly referred to as setback region. The difference between the two ends of this region is known as the **setback viscosity**. The re-association between starch molecules during cooling is commonly referred to as setback. It involves retrogradation, or re-ordering, of the starch molecules, and has been correlated with texture of various products. High setback is associated with syneresis, or weeping, during freeze/thaw cycles.

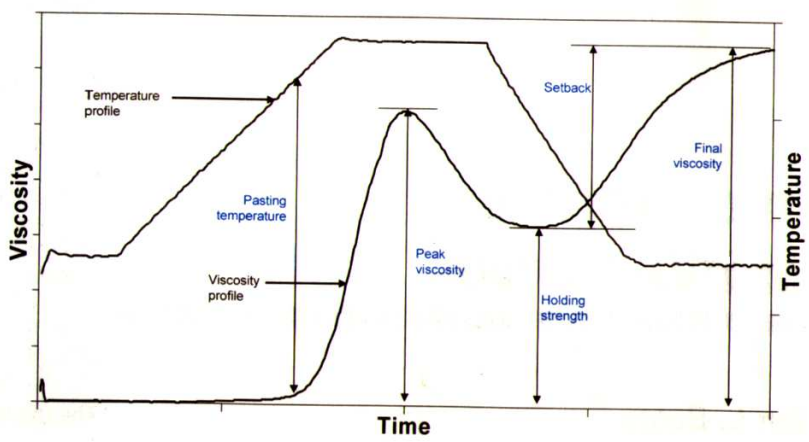


Fig. 1 Typical complete RVA curve, showing the main parameters used to describe the pasting.

Rapid Visco Analyzer:

Rapid Visco Analyzer (RVA) (Fig.1) is a rotational viscometer that is able to continuously record the viscosity of a sample under conditions of controlled temperature. The ability of the RVA to suspend samples in a solvent, maintain them in suspension throughout the test, and apply an appropriate degree of shear to much processing conditions makes it

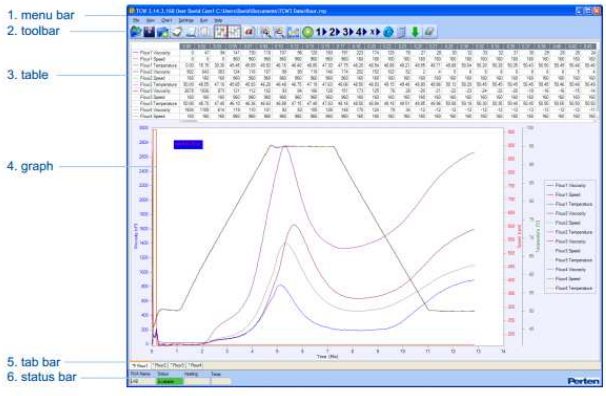
particularly valuable in many process and research applications. It is especially useful for testing materials that require relatively complex test routines, using varying temperature and shear, allowing materials to exhibit their full range of viscous properties. The combination of shearing, heating and cooling, applied over time, creates a viscosity curve for the material. Testing a starch sample using your RVA typically involves heating and cooling the starch sample in water and monitoring its viscosity. The shape and size of the pasting curve for a particular starch sample will depend on the properties of that starch.

Fig.1 Rapid Visco Analyzer (RVA)

The instrument was combined with the Thermocline for Windows (TCW) software programme, is able to heat and cool samples through more than 100 temperature ramps, over periods ranging from seconds to, in the case of model RVA-4, more than 100 hr. This versatility means that the RVA can be applied to the testing of a wide range of materials and can extract information that might not be uncovered using alternative techniques. The combination of shearing, heating, and cooling, applied over time, creates a viscosity curve for the material that shows a characteristic number of inflections, peaks, and troughs. The TCW software is able to capture the desired characteristics of the curve and provide a table of values about that sample. Alternatively, curves from different samples may be compared manually by overlaying them on a computer screen.

The main window of TCW consists of:

1. A menu bar accessing most program functions.
2. A toolbar for easily accessing commonly used functions.
3. A table of test data.
4. A graph for graphically displaying test data.
5. A tab bar for selecting which data set to view of those in the currently open project.
6. A status bar displaying the current program status and information.



METHODS OF DETERMINATION

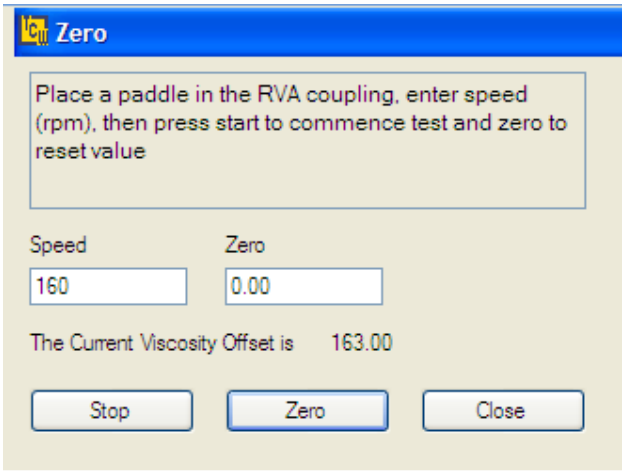
Sample preparation

Good sample preparation is essential for obtaining consistent and accurate results from your RVA-4. If sample is of whole grain, it should be ground using a hammer mill. Flour and whole meal do not require any milling. The moisture content of the sample should be measured to enable moisture correction. Samples should be prepared in accordance with local operating procedure. weigh and dispense the sample material and water into a new canister. Place a new paddle into the canister and jog the blade vigorously up and down through the water 10 times. Ensure any sample lumps adhering to the inside of the canister are pushed down into the water.

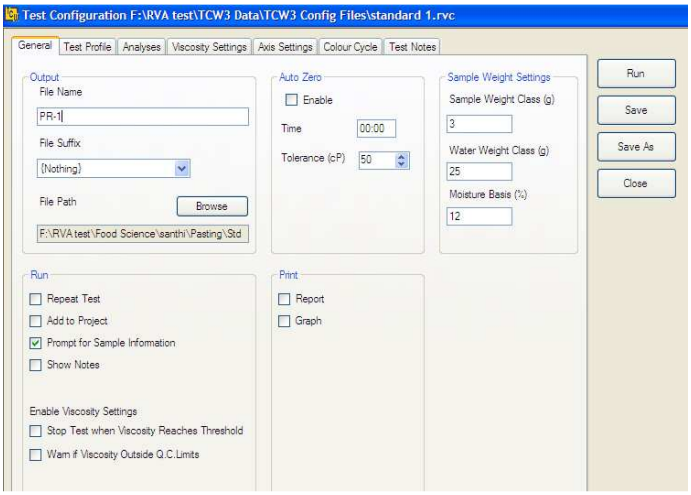
Steps in determination

The steps in determination of pasting properties are,

- Switch on the RVA- Techmaster, zero the viscosity of the instrument by loading only paddle.



- Load the canister with paddle containing sample under test.
- Create a new test configuration file or Edit a test configuration file or Run last test.
- Enter the required details under each tab of test configuration file



- Save the test configuration file.

- Run the test under selected protocol in test configuration file.
- View and analyze test results.
- Generate the reports.

Profiles for determination

The starting point for performing any RVA analysis is to decide what sequence of paddle rotation speeds and temperature to which the sample is subjected. This sequence is called a "profile". It comprises a series of timed (hours: minutes: seconds) targets of paddle speed (rpm) and temperature (˚C). Profiles may be created from scratch or modified to fit a particular need. When working with conventional materials or where data need to be shared, RVA users adopt standardized profiles rather than develop their own slightly different profile. Of particular note are the AACC, ICC, and RACI standard methods, as well as the manufacturer-provided "Standard 1" and "Standard 2" profiles, which are widely used for simple applications (Table 1).

Table 1: Stages of RVA Standard Profile 1 and Profile 2

S. No.	Stage	Profile 1	Profile 2
1	Initial temperature, ◦C	50	50
2	Initial holding time, min	1	2
3	Heating time, min	3 min, 42 sec	7 min, 30 sec
4	Maximum temperature, ◦C	95	95
5	Hold at max. temp., min	2 min,30 sec	5 min, 30 sec
6	Cooling time, min	3 min,48 sec	4
7	Final temperature, ◦C	50	50
8	Final holding time, min	2	4
9	Total test time, min	13	23

The optimized profile for determination of pasting properties of rice flour/starch by different authors are indicated in Table 2.

Table 2: Optimized protocol for determination of pasting properties of rice flour/starch

S. No.	Sample Name	Protocol For Pasting Properties	Parameters Assessed	Equipment	References
1.	Rice flour	The sample (3 g of flour on 12% moisture basis) was mixed with 25 mL of deionized water in RVA sample canister. The idle temperature was set at 50˚C and the following 12.5 min set profile was run: 50˚C held for 1.0 min, the temperature was linearly ramped up to 95˚C until 7.3 min, the temperature was linearly ramped down to 50˚C at11.1 min and held at 50˚C until 12.5 min.	Peak viscosity, hot past viscosity, cold past viscosity, breakdown and setback viscosity	Rapid Visco Analyzer (RV, model-4)	Kim, C. & Yoo, B. (2006)

2.	Rice flour	Flour (3.5g db) was weighed into an RVA canister followed by addition of silver nitrate solution (0.021M) to achieve a final net weight of 29 g. The flour suspension was analyzed under continuous shear (160 rpm) beginning with an initial hold at 60˚C (2min), linear heating to 95˚C (5min), an intermediate hold at 95˚C (4 min), linear cooling to 50˚C (5 min), and a final hold at 50˚C (4 min) to yield a total test time of 20 min.	Peak viscosity, break down viscosity, trough, final viscosity, setback viscosity	RVA	Batey, L., & Curtin, B.M. (1997)
3.	Glutinous rice flour	Weighed 3 g of rice flour into a Rapid Visco Analyzer (RVA) canister and added with 25 ml of distilled water with and without amylase inhibitor (10mMCuSO4). The mixture was subjected to RVA analysis with a constant stirring rate (160 rpm) and temperature (37˚C) for 1 hr.	Peak viscosity, breakdown, final and set back viscosity.	RVA super 3, Newport Scientific, New South Wales, Australia).	Surojanametakul, V. & T.Yoshihashi. (2003)
4.	Red rice flour	3 g of flour (14%wet basis) sieved through a 250 µ mesh were suspended in distilled water to complete 28 g. The viscosity profile was obtained according to the following time/temperature regime: 25 °C for 2 min, heating from 25 to 95 °C at a rate of14°C/min; holding the paste at 95°C for 3 min (heating cycle); cooling from 95 to 25°C at a rate of 12°C/min.	peak viscosity, maximum peak viscosity, breakdown viscosity.	RVA (Rapid Visco Analyzer 4.	Ascheri, D.P.R., Andrade, C.T., Carvalho, C.W.P., & Ascheri J.L.R. (2006)
5.	Rice flour	Rice flour (3 g on dry basis) was poured into distilled water (25 ml) in a canister and mixed thoroughly. The mixture was stirred at 960 rpm for 10 sec and then changed to 160 rpm. Its temperature was first maintained at 50˚C for 1.5 min and then raised to 95˚C at a rate of 12˚C/min. After that the temperature was maintained at 95˚C for 2.5 min, followed by a cooling down to 50˚C at 12˚C/min and was maintained at 50˚C for 2.1 min.	Peak viscosity, temperature at peak viscosity, trough, final viscosity, the breakdown viscosity, and setback viscosity.	RVA	Rordprapat Wathanyoo, Nathakaranakule, Adisak, Tia, Warunee, Soponronnarit., & Somchart. (2005)
6.	Rice flour	8% of rice flour slurry (~40 g, corrected to 14% of moisture in 420 ml of deionized water) was heated from 50 to 95˚C for 15 min, and cooled at 50˚C at 1.5˚C/ min and finally held at50˚C for 15 min.	Peak viscosity, trough, breakdown, final viscosity, setback viscosity, peak time, pasting temperature.	Bra bender viscograph-E (Brabender OHG, Dusisburg, Germany)	Gayin, J., Manful, J.T., & Johnson, P-NT. (2009)
9.	Rice starch	Rice starch slurry alone (6% w/w)	Peak viscosity,	RVA	Viturawon

		was prepared by dispersing weighed amount of rice starch (dry basis) in distilled water or salt solutions, the slurries were stirred for one minute at room temperature to avoid lump formation. The slurries (28 g) poured in canister. The heating and cooling cycles were programmed, according to standard profile 1. The slurry was held at 50˚C for one min, heated to 95˚C within 3 min 42sec and then held at 95˚C for 2 min 30sec. it was subsequently cooled to 50˚C within 3 min 48sec and held at 50˚C for 2 min at rotation speed of 160 rpm.	breakdown viscosity, set back viscosity and pasting temperature		g, Y., Achayutha kan, P., & Suphanthar ika, M. (2008)
13.	Brown rice (Japonica type, Ilpum)	Flour slurries (15% w/w, db, 30 g total weight) were held at 50˚C for 1 min, heated to 95˚C at a rate of 12.2 ˚C/min, held at95^{o}C for 2.5 min, cooled to 50˚C at 12.2 ˚C/min, and held at 50˚C for 2 min.	Pasting temperature, peak viscosity, breakdown, setback and final viscosity.	RVA	Chung, H.J., Cho, A. & Lim, S.T. (2012)
14.	Hydrother mally treated Rice flour	A 13.8% (w/w) rice flour suspension was prepared by placing 4 g (dry basis) rice flour in an aluminium canister which contained 25 g of distilled water. A programmed heating and cooling cycle was used at constant shear rate, where the sample was equilibrated at 35°C for 2 min, heated to 95°C at a rate of 11.8°C/min, held at 95°C for 2.5 min and cooled to 35°C at the same rate. (Approved method 61-02 AACC, 1995)	Cold past (initial) viscosity, peak viscosity (PV), temperature at PV (P_{temp}), final viscosity (FV), breakdown viscosity (BKD=PV–trough), and total setback viscosity (TSB=FV–trough)	Rapid Visco Analyzer model 3-D	Lai, H.M. (2001

Factors to be considered when developing RVA methods

The ability of the RVA user to vary sample preparation methods, concentration, temperatures, and shear conditions makes RVA analysis effective in an enormous range of applications. However, the range of test profiles made possible by this flexibility can be overwhelming when first considering the use of the RVA for a chosen application. Nonetheless, despite the abundance of possible methods, RVA method development can proceed very quickly since test parameters are quickly and conveniently changed. It is valuable to be aware of factors that are already known to impinge on viscosity profiles when developing RVA analysis methods and some of these factors are exceedingly simple.

Time

Time is an important consideration in profile creation. The effects of temperature and shear rate vary according to the period of time they are applied. Chemical reactions, enzyme reactions, and physical interactions all need time to proceed. The effect of any of these can be

amplified by extending appropriate conditions for longer times. Additionally, when applying temperature changes, remember that they take time to occur and are limited by the rate at which heat energy can add to, or removed from, the sample. The maximum specified heating rate in current commercially available RVA model is 14°C per minute. This rate assumes that there is no boiling of the solvent. The maximum cooling rate is determined by coolant temperature and flow rate but is typically somewhat faster than heating. Furthermore, heat transfer through the liquid sample is limited by the dimensions of canister and paddle, paddle rotation rate, apparent viscosity of the fluid, and boundary layer effects at the walls of the canister (Rao, A.M. 1999). For the most precise determination of viscosities at particular temperature, profile must allow sufficient time for temperatures through the mass of the fluid. In some cases, time to stabilize viscosity is a trade – off with the degradation of the sample caused by extended periods of stirring at elevated temperatures. In these cases, only indications, rather than absolute viscosity results, can be obtained.

Temperature

The temperature range of commercially available RVA is 0-100°C. These limits are set by external physical constraints. Samples mixed in water boil at 100°C at sea level, causing complete distruption of the fluid under test and preventing any measurement of viscosity. Similarly, reducing sample temperatures to below room temperature requires a refrigerated coolant supply, and practical considerations such as heat flow from ambient conditions, condensation, and freezing of components restrict low-temperature testing (www.food safety.gov.1999). The specific heat stored in the RVA heating block at the idle temperature can provide valuable heat energy to rapidly bring samples to the desired temperatures at the beginning of a test. This technique can also be described as "ballistic" heating (or cooling).

Temperature ramping rates

Temperature ramping rates are established by setting the time and temperature targets by using the TCW software. In RVA profile, "time" is the total elapsed time since the beginning of the test. As the test progresses, the RVA adjusts the sample temperature in a linear ramp (up and down) from target to target. The temperature ramp rate (or slope of the line between the targets) should be considered when developing profiles.

Particle size effects

A longer time is required for larger particles to be fully wet by the solvent and for heat energy to flow into, or out of, the particles. Wetting time is also affected by the nature of the particles. Friable particles usually have micro-structures such as cracks or porosity that accelerate solvent ingress. Hence absolute size, size distribution and sample preparation affect RVA results and must be controlled to obtain consistent data.

Shear rate

The effective shear rate generated by the RVA paddle rotating in the canister is approximately 0.34 sec^{-1}/rpm. For historical reasons, many widely used RVA profiles use a paddle rotation speed of 160 rpm, providing an effective shear rate of 54 sec^{-1}. Shear rate selection in a profile should consider that most food biopolymers exhibit shear thinning behavior (Rao, A.M. 1999). The flexibility of RVA temperature and shear profiling allows

users to tailor test routines to those best suited to their experimental or process control needs.

Water quality, solvents and Buffers

Majority of RVA test procedures use water or an aqueous buffer as the solvent. This is largely because food systems are, in the main, water based, particularly those employing starches. It is very important to recognize, however, that water "quality" can have significant effect on RVA results. Even stale water from water deionizing units has been found to be the cause of irregular data. Water should comply with ISO standard 3696 or a similar standard. Using more volatile-solvents restricts operating temperature. Solvents that may generate expensive gases should never be used in the RVA. Many industrial starches require testing in alkaline solutions of pH 8-12 (Mauro, D.J., Abbas, I.R., and Orthoefer, F.T. 2003). High - pH reagents attack the aluminum canisters rapidly, changing the chemistry and emitting gas bubbles that interfere with the measurement of viscosity. Specially coated canisters, available from Newport Scientific, are able to withstand these conditions. The coating very slightly reduces the flow of heat into canister contents, which may affect determinations of pasting temperature compared with those from noncoated canisters.

Evaporation

The RVA canisters are not sealed. At elevated temperatures, solvents (including water) evaporate from the mixture being tested. The evaporation rate depends on the difference between sample vapor pressure and ambient vapor pressure. In longer tests, i.e., more than 20 min duration particularly at elevated temperatures, results certainly are affected to some degree, and due care should be applied in controlling or monitoring this effect.

Cavitation

In some cases, air bubbles can form behind the paddle blades, leading to artificially low viscosity readings. As the bubbles form and collapse, the viscosity trace falls or jumps, giving a jagged or sawtooth curve. Pregelled and waxy starches are more prone to cavitation. Cavitation problems can usually be overcome by increasing the total canister contents, working at lower concentratoins, lowering paddle speeds, or even removing the initial fast-mix step from a test profile. All of these approaches reduce the chance of a vortex forming around the paddle shaft, which can entrap an air bubble behind the paddle.

Effects of other components

Protein or lipid interact with the starch and affect it measured viscosity. α- amylase reduce the viscosity by digesting the starch during the test. Damaged starch present in the flour or extruded products result in "cold swelling" and affect the size of the peak. Flours with greater amounts of starch damage show larger peaks. Addition of lipids usually reduces the peak viscosity but have little effect on final viscosity. Rice pasting peak has been shown to increase with increased degree of polishing (Perdon, A.A., Siebenmorgen, T.J., Mauromoustakos, A., Griffin, V.K., and Johnson, E.R. 2001). The method used to grind rice to flour has also been shown to markedly affect the pasting of waxy rice (Chen, J. J. Lu, S. Lii. C. Y. 1999), and particular care should taken when grinding rice for RVA analysis because it has very hard endosperm. Gentler grinding and sieving is recommended when testing more fragile (e.g., pre-cooked) samples (Becker, A., Hill, S.E., & Mitchell, J.R., 2001). Dense

particles from ground rice are also prone to settle out of suspension when mixing at low speeds (<100 rpm) (Blakeney, A.B. 1996).

Application

The RVA has been used successfully to assess the effects of cultivar, growth environment, harvest time, grain storage, grain constituents, endosperm hardness, formulation, milling, chemical modification, cooking, drying, and shelf storage on all of the major cereal grains and their derivatives. This wide range of applications is testimony to the versatility of the instrument and its applicability in this field. As a tool for the researcher, the RVA provides a "window" for investigating grain quality and the factors affecting it. Cereal breeders have taken advantage of the small sample size and rapid test cycle for early-generation selection of favorable lines. For traders and regulators, RVA methods are used to classify grain and ensure conformance with agreed-upon specifications. Processors can use it to screen and modify ingredients and as a tool to monitor and adjust their process to improve product consistency and quality.

CONCLUSION

Pasting properties are among the most important characteristics of starch, determining its applications in food processing and other industries. In the food industry, the RVA is a good tool for obtaining information related to apparent viscosity. Pasting properties have been used to predict the end-use quality of various products, for example cooked rice texture and noodles. The pasting profile shows the physicochemical changes of the starch aqueous suspension as a function of temperature and time, and allows the determination of the apparent viscosity of the sample under study. The peak time and peak viscosity can be associated with the water absorption capacity. Peak and final viscosities of a sample are important parameters to consider in product development, as they give an idea of how the product may behave during and after processing. High setback values are correlated with the amylose content, and even higher setback values are considered to be an indicator of better palatability. The viscosity of a paste depends on a large extent on the degree of gelatinization of the starch granules and the extent of their molecular breakdown. The decrease in magnitude of the peak viscosity and final viscosity might reflect greater degradation and gelatinization of starch. Thus the RVA is used to simulate food processing and to related functionality and structural properties.

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Phenolic Compounds: Successful Substitute for Lipid Retardation.

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ABSTRACT

Spoilage prevention and shelf-life extension of products will always remain an important goal to the meat industry. The auto-oxidation of fats is a big problem because of the deterioration in the quality of the foods in which they are contained and the reduction in their nutritional value. Fatty fish contains high concentration of polyunsaturated fatty acids (PUFA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and therefore, fish and fish products are susceptible to loss of quality through lipid oxidation. Safeguarding fats against oxidation is normally done by restricting the access of oxygen or adding antioxidants. The most commonly applied antioxidants are synthetic phenols, such as, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). The use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences. Natural phenolic compounds with antioxidant activity such as grape seed extrac, rosemary extract, tea, catechin and tannins etc have been gaining increasing attention due to their safety. Therefore, attention is focused on natural antioxidants. These antioxidants are polyphenol compounds, which are found in all plants and in all parts of the plants (tree bark, stalks, leaves, fruits, roots, flowers and seeds).

KEYWORDS: Spoilage**,** Lipid oxidation, Antioxidants, Phenolic compounds,

INTRODUCTION

Dietary lipids, naturally occurring in raw food materials or added during food processing, play an important role in food nutrition and flavor. Meanwhile, lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. As far as Fish lipids are concerned, it is an important source of nutritional components due to having high content of essential long-chain omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which protect against heart disease, cancer, hypertension, rheumatoid arthritis, depression, diabetes, among other health benefits. These findings have influenced the increasing demand for n - 3 PUFA rich fish oils and foods enriched by these oils (Medina *et al*., 2012)**.** The American Heart Association generally recommends a daily intake of n-3 PUFA up to 400–500 mg EPA and DHA, which may be reached by consuming at least two servings of preferably oily fish per week. Due to this high content of PUFAs in fish muscle, as well the presence of relevant pro-oxidant compounds, promote the development of enzymatic and non-enzymatic rancidity leading to changes in the quality attributes of fish and fisheries product including taste, texture, shelf life, appearance and flavor due to the formation of

harmful components including free radicals and reactive aldehydes (Conde *et al.*, 2011). In addition, the oxidation of polyunsaturated fatty acids in biological membranes leads to serious damage such as coronary atherosclerosis, emphysemas, cancer and cirrhosis. Apart from serious damage, lipid oxidation will lead to formation of toxic oxidation products and these toxic oxidative products can be minimized by using the effective antioxidant. An antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals which can perpetuate the reaction. Antioxidants can bind metals, scavenge species that initiate or perpetuate oxidation, quench high energy oxygen species preventing formation of peroxides, or decompose lipid peroxides. The antioxidants are of synthetic ones or from the natural source and the use of synthetic antioxidant have been widely employed to retard lipid oxidation in food. However, the use of synthetic antioxidants has raised questions regarding food safety and toxicity. The use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences. Natural phenolic compounds with antioxidant activity such as grape seed extract, papaya seed extract, rosemary extract, tea, catechin, tannins, etc. have been gaining increasing attention due to their safety (Maqsood and Benjakul, 2010).

Lipid oxidation mechanism

Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives. Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species. Among these, only autoxidation and thermal oxidation are discussed here in detail. Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids (Gordon *et al*., 2001). The process can be accelerated at higher temperatures, such as those experienced during deep-fat frying, which is called thermal oxidation, with increases in free fatty acid and polar matter contents, foaming, color, and viscosity . Unsaturated fatty acids are generally the reactants affected by such reactions, whether they are present as free fatty acids, triacylglycerols (as well as diacyglycerols or monoacylglycerols), or phospholipids.It has been accepted that both autoxidation and thermal oxidation of unsaturated fatty acids occurs via a free radical chain reaction that proceeds through three steps of initiation, propagation, and termination (Kamal-Eldin *et al*., 2003).

- **Initiation**

  $In \cdot + LH$ ------> $InH + L \cdot$ (alkyl free radical)
- **Propagation**

  $L \cdot + O_2$ ------> $LOO \cdot$ (peroxy radical)

  $LOO \cdot + LH$ ------> LOOH (hydro peroxide) + $L \cdot$
- **Termination**

  $LOO \cdot + LOO \cdot$ ------> LOOL (peroxides) + O_2

METHODS FOR MEASURING LIPID OXIDATION

Numerous analytical methods are routinely used for measuring lipid oxidation in foods. However, there is no uniform and standard method for detecting all oxidative changes in all food systems. Therefore, it is necessary to select a proper and adequate method for a

particular application. The available methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products (Dobarganes and Velasco 2002). A number of physical and chemical tests, including instrumental analyses, have been employed in laboratories and the industry for measurement of various lipid oxidation parameters. These include the weight-gain and headspace oxygen uptake method for oxygen absorption; chromatographic analysis for changes in reactants. iodometric titration, ferric ion complexes, and Fourier transform infrared (FTIR) method for peroxide value; spectrometry for conjugated dienes and trienes, 2-thiobarbituric acid (TBA) value, p-anisidine value (p-AnV), and carbonyl value; Rancimat and Oxidative Stability Instrument (OSI) method for oil stability index; and electron spin resonance (ESR) spectrometric assay for free-radical type and concentration. Other techniques based on different principles, such as differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR), have also been used for measuring lipid oxidation. In addition, sensory tests provide subjective or objective evaluation of oxidative deterioration, depending on certain details.

MEASUREMENT OF PRIMARY PRODUCTS OF OXIDATION

Peroxide Value (PV)

Lipid oxidation involves the continuous formation of hydroperoxides as primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products. The formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Therefore, the peroxide value (PV) is an indicator of the initial stages of oxidative change (Riuz *et al*., 2001).

Conjugated Dienes and Trienes

It was discovered in 1933 that the formation of conjugated dienes in fats or oils gives rise to an absorption peak at 230–235 nm in the ultraviolet (UV) region. In the 1960s, monitoring diene conjugation emerged as a useful technique for the study of lipid oxidation. During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. The resulting conjugated dienes exhibit an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm. An increase in UV absorption theoretically reflects the formation of primary oxidation products in fats and oils (Shahidi *et al*., 1994).

MEASUREMENT OF SECONDARY PRODUCTS OF OXIDATION

The primary oxidation products (hydroperoxides) are unstable and susceptible to decomposition. A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation (Kamal-Eldin *et al*.,). Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, among others. Methods for assessing lipid oxidation based on their formation are discussed in this section

Thiobarbituric Acid (TBA) Test

The thiobarbituric acid (TBA) test was proposed over 40 years ago and is now one of the most extensively used methods to detect oxidative deterioration of fat-containing foods (Kishida *et al*., 1993). During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids with 3 or more double bonds, is formed as a result of the degradation of polyunsaturated fatty acids. It is usually used as an indicator of the lipid oxidation process, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method (Cesa 2004). In this assay, the MA is reacted with thiobarbituric acid (TBA) to form a pink MA-TBA complex that is measured spectrophotometrically at its absorption maximum at 530–535 nm (Antolovich *et al*., 2002).

p -Anisidine Value (p -AnV)

The p-anisidine value (p-AnV) method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. It is based on the color reaction of p-methoxyaniline (anisidine) and the aldehydic compounds. The reaction of p-anisidine reagent with aldehydes under acidic conditions affords yellowish products that absorb at 350 nm. The color is quantified and converted to p-AnV. The p-AnV is defined as the absorbance of a solution resulting from the reaction of 1 g of fat in isooctane solution (100 ml) with p-anisidine (0.25% in glacial acetic acid) . This test is more sensitive to unsaturated aldehydes than to saturated aldehydes because the colored products from unsaturated aldehydes absorb more strongly at this wavelength. However, it correlates well with the amount of total volatile substances. The p-AnV is a reliable indicator of oxidative rancidity in fats and oils and fatty foods (Van der Merwe *et al*., 2003).

Totox Value

The Totox value is a measure of the total oxidation, including primary and secondary oxidation products. It is a combination of PV and p-AnV (Stauffer 1996).

$$\text{Totox value} = 2\text{PV} + \text{p-AnV}$$

ANTIOXIDANT AND ITS MECHANISM

In foods, antioxidants are defined as substances that in small quantities are able to retard or to prevent the oxidation of oxidizable materials such as fats. Antioxidants are food additives that retard atmospheric oxidation and its degrading effect thus extending the shelf life of food (Somogyi, 2006). These substances can occur as natural constituents of foods, but they also can be intentionally added to products or formed during processing. Their role is not to enhance of improve the quality of foods, but they do maintain food quality and extend shelf life. Antioxidants for use in food processing must be inexpensive, nontoxic, effective at low concentrations, stable, and capable of surviving processing (carry-through effect); color, flavor, and odor must be minimal. The choice of which antioxidant to use depends on product compatibility and regulatory guidelines.

Antioxidants can be broadly classified by mechanism of action as:

a) Primary antioxidants
b) Secondary antioxidants.

The primary or chain breaking antioxidants can react with lipid radicals to convert them into more stable products, while the secondary or preventive antioxidant can reduce the rate of lipid oxidation by a variety of mechanisms.

a) Primary (chain-breaking) antioxidant

A primary antioxidant, also known as "chain-breaking" antioxidant, is a substance that can accept free radicals and further delay the initiation step or interrupt the propagation step of autoxidation. Primary antioxidants (AH) can react with lipid and peroxyl radicals and convert them into more stable radicals or non-radical products as shown in the following equations(Kamal-Eldin *et al*.,2003).

$$R\bullet + AH \rightarrow RH + A\bullet$$
$$RO\bullet + AH \rightarrow ROH + A\bullet$$
$$ROO\bullet + AH \rightarrow ROOH + A\bullet$$

The antioxidant radicals (A•) produced by this process are much less reactive than lipid or peroxyl radicals, and therefore do not promote oxidation as lipid or peroxyl radicals do. These antioxidants radicals, in fact, can also terminate the lipid oxidation reaction by reacting with peroxyl radicals, alkoxyl radicals and other antioxidants as shown in the following equations.

$$RO\bullet + A\bullet \rightarrow ROA$$
$$ROO\bullet + A\bullet \rightarrow ROOA$$
$$A\bullet + A\bullet \rightarrow AA$$

b) Secondary antioxidant

Secondary antioxidants can retard lipid oxidation through a variety of mechanisms, including chelation of transition metal ions, oxygen scavenging, replenishing hydrogen to primary antioxidants, absorbing UV radiation and deactivation of reactive species. The main difference between primary and secondary antioxidants is that secondary antioxidants do not transform or convert free radical species into more stable products. Secondary antioxidants usually only delay oxidation by interfering with the prooxidant system, such as metals, radiation etc. Many of them show antioxidant activity only if a minor prooxidative component is present in the system. For instance, sequestering agents are only effective in presence of metal ions, and reducing agents such as ascorbic acid are effective in presence of tocopherols or other phenolic antioxidants (Kamal-Eldin *et al*., 2003).

TYPES OF ANTIOXIDANTS

i) *Synthetic antioxidant*
ii) *Natural antioxidant*

i) *Synthetic antioxidant*

Some of the most used synthetic antioxidants are phenolic compounds such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG). They are used widely in the food industry because of their effectiveness and generally being less expensive than natural antioxidants. Concerns

regarding toxicological effects and carcinogenic potential of synthetic antioxidants have prompted the need for natural alternatives in the last few decades. Since about 1980, natural antioxidants have appeared as a healthier and safer alternative to synthetic antioxidants (Yanishlieva, 2001).

ii) *Natural antioxidant*

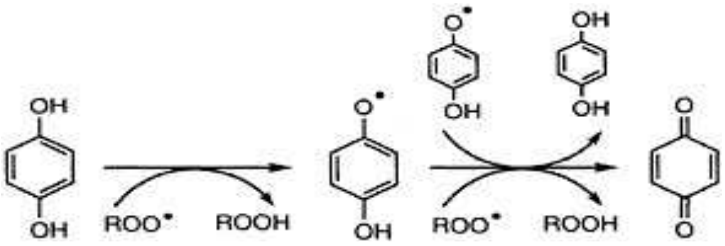
Due to the increasing concerns regarding safety issues of using synthetic antioxidants, research has focused on the development and utilization of antioxidants from natural sources. The empirical use of natural compounds as antioxidants is very old. The popularity of smoking and spicing in the home for preservation of meat, fish and other fat-rich foods may have be due to the recognition of the rancidity-retarding effect of these treatments (Yanishlieva, 2001). Natural antioxidants are found in almost all plants, microorganisms, fungi, and even in animal tissues. The majority of natural antioxidants are phenolic compounds, and the most important groups of natural antioxidants are the tocopherols, flavonoids and phenolic acids. The mechanisms of these natural antioxidants on autoxidation control or rancidity prevention may be different. However, their presence in live plants may be for the sake of protecting tissues from injurious damage. Furthermore, the beneficial effects of consuming plant food have been ascribed, at least in part, to the presence of antioxidants in the plant and are associated with lowering the risk of most cardiovascular diseases and cancer, among other degenerative diseases of aging (Gordon, *et al.,*).

Phenolic compounds as antioxidants

In a biological system, an antioxidant can be defined as ''any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate''. The oxidizable substrate may be any molecule that is found in foods or biological materials, including carbohydrates, DNA, lipids, and proteins. Food is a multi component system composed of a variety of biomolecules, and therefore, this definition describes well an antioxidant. Natural antioxidants from dietary sources include phenolic and polyphenolic compounds. The mechanism by which these antioxidants exert their effects may vary, depending on the compositional characteristics of the food. Furthermore, the beneficial health effects of consuming plant foods have been described, in part, to the presence of phenolics, which are associated with counteracting the risk of cardiovascular diseases, cancer and cataract as well as a number of other degenerative diseases. This is achieved by preventing lipid oxidation, protein cross linking and DNA mutation and, at later stages, tissue damage. Although, phenolic compounds and some of their derivatives are very efficient in preventing autoxidation, only a few phenolic compounds are currently allowed as food antioxidants. The major considerations for acceptability of such antioxidants are their activity and potential toxicity and/or carcinogenicity (Shahidi,1994).The approved phenolic antioxidants have been extensively studied, but the toxicology of their degradation products still is not clear. Some commercially produced plant phenolic compounds have recently been considered as antioxidants. Phenolic compounds such as tea catechins, grape procyanidins, rosemary extracts and olive oil hydroxytyrosol have been found to retard lipid oxidation in fish muscle-based food products. Even though many natural and synthetic compounds have antioxidant properties, only a few of them have been accepted as "generally recognized as safe" (GRAS) substances for use in food products by international bodies such as the Joint FAO/WHO Expert Committee for Food Additives (Cesa, 2004).

Antioxidative action of phenolic compounds

Phenolic antioxidants (AH) interfere with lipid oxidation by rapid donation of a hydrogen atom to lipid radicals. The reaction of antioxidant and lipid radical is exothermic in nature. The activation energy increases with increasing A-H and R-H bond dissociation energy. Therefore, the efficiency of the antioxidants increases with decreasing A-H bond strength (Gordon, 2001). The resulting phenoxy radical must not initiate a new free radical reaction or be subjected to rapid oxidation by a chain reaction. In this regard, phenolic antioxidants are excellent hydrogen or electron donors.



SCOPE OF USING ANTIOXIDANTS IN FOOD

The function of an antioxidant is to retard the oxidation of an organic substance, thus increasing the useful life or shelf life of that material. In fats and oils, antioxidants delay the onset of oxidation or slow the rate of oxidizing reactions. Oxidation of lipids chemically produces compounds with different odors and taste and continues to affect other molecules in the food. The main purpose of using an antioxidant as a food additive is to maintain the quality of that food and to extend its shelf life rather than improving the quality of the food. Use of antioxidants reduces raw material wastage and nutrition loss and widens the range of fats that can be used in specific products. Thus, antioxidants are useful additives that allow food processors to use fats and oils economically in their product formulation (Kishida, 1993).

EXTRACTION METHOD OF NATURAL ANTIOXIDANTS

1) Liquid-liquid extraction (LLE)
2) Solid-liquid extraction (SLE)
3) Super-fluid extraction (SFE)

1) *Liquid-liquid extraction (LLE):* Liquid-liquid extraction is a mass transfer operation in which a liquid solution (the feed) initially containing one or more solutes is thoroughly mixed with an immiscible or nearly immiscible liquid (solvent). It is an extraction of substance from one liquid phase into another liquid phase. The solvent exhibits preferentially affinity or selectivity towards one or more of the components in the feed and has different density. Two stream results from this contact: the extract, which is the solvent rich solution containing the desired extracted solute and the raffinate, the residual feed solution containing little solute (Tomsone, *et al.,* 2012).

For the separation of phenolic compounds, liquid–liquid extraction is frequently used with industrial liquid by-products, such as those resulting from the beverage industry (grape, orange juices, wines etc.)

2) *Solid-liquid extraction (SLE):* Solid–liquid extraction or leaching can be defined as a mass transport phenomenon in which solids contained in a solid matrix migrate into a solvent brought into contact with the matrix. Mass transport phenomena can be enhanced by changes in concentration gradients, diffusion co-efficients or boundary layer. It is a unit operation extensively used to recover many important food components: sucrose in cane or beets, lipids from oilseeds, proteins in oilseed meals, phytochemicals from plants, functional hydrocolloids from algae and polyphenolic compounds from plants, fruits, vegetables etc (Tomsone, *et al.,* 2012).

The fundamental principle behind leaching is the removal of a soluble material from an insoluble, permeable solid phase. The soluble fraction, solid or liquid, may exist mechanically in the pore structure of the insoluble material or chemically combined with that material. This soluble material is removed through dissolution in a dissolving solvent. The most familiar example of leaching is the extraction of tea and coffee, and most importantly mineral recovery (Tomsone, *et al.,* 2012).

3) *Super-fluid extraction (SFE):* Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). Carbon dioxide (CO2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present. Supercritical Fluid Extraction (SFE) is based on the fact that, close to the critical point, the solvent changes its properties rapidly with only slight variations of pressure (Tomsone, *et al.,* 2012).

SFE methods are rapid, automatable, selective and avoid the use of large amounts of toxic solvents. In addition, the absence of light and air during the extraction reduces the degradation processes that can occur during the traditional extraction techniques. In natural product extraction and isolation, supercritical fluid extraction (SFE), especially that employing supercritical CO_2, has become the method of choice (Tomsone, *et al.,* 2012). Sophisticated modern technologies allow precise regulation of changes in temperature and pressure, and thus manipulation of the solvating property of the SCF, which helps the extraction of natural products of a wide range of polarities. By adding modifiers to a SCF (like methanol to CO_2) its polarity can be changed for obtaining more selective separation power. Therefore, supercritical carbon dioxide (SC–CO_2) methods are ideal for the extraction of natural products from plant materials and are particularly recommended for the extraction of thermolabile compounds, when low temperatures are required. In addition, SC–CO_2 methods allow obtaining extracts without remaining solvent traces and without using a cleaner, as the degradation of certain compounds by lengthy exposure to high temperatures or oxygen is avoided (Tomsone, *et al.,* 2012).

SOLVENT EXTRACTION

Extraction can be defined as the removal of soluble components from solid or liquid materials by means of an immiscible solvent. In the preparation of a sample for analysis, it is common practice to first extract the analyte away from the bulk of the matrix material and

then to remove potentially interfering coextractives by one or more cleanup steps. In the simplest application, it is desired to remove some solute or group of solutes from an insoluble matrix (e.g., animal or plant tissue) (Tomsone, *et al.,* 2012).

Solvent extraction is defined as the process of separating one constituent from a mixture by dissolving it into a solvent, in which it is soluble while the other constituents of the mixture are not, or are at least less soluble (Holden, 1999). Solvent extraction is one of the oldest and most widely used techniques in the preparation of samples for qualitative and quantitative analysis. In its simplest form, extraction has been used since ancient times. The technique helps scientists to remove as much of the analyte from the matrix as practical, and with a minimum extraction of extraneous materials that might interfere in the analysis if the proper solvent is chosen and used during the process (Tomsone, *et al.,* 2012).

There are three reasons for using solvent extraction:
(1) To isolate a component or analyte of interest
(2) To remove potential interferents from a matrix
(3) To preconcentrate an analyte prior to measurement

The very basic principle of solvent extraction is "like dissolves like". Removing a non-polar constituent from a sample matrix requires employing non-polar solvents. If the solute is charged, it is usually best to form an ion-pair with a counter ion and extract the newly formed neutral complex into a non- polar solvent. Likewise, a polar solvent should be used in the extraction process in order to remove a polar solute from a solution. The major problem in extracting polar solutes into polar solvents is the miscibility of polar solvents with water, which is the main matrix for many samples (Tomsone, *et al.,* 2012).

Qualitative predictions could be made for an extraction by considering the polarity of the desired analyte and of the solvents used. The solvent should be able to provide the highest solvency for the solutes of interest, and this solvent should also be matched in terms of polarity and selectivity to the solutes. Thus, the extraction of hydrophilic samples such as animal tissue is best done with water-miscible solvents such as methanol or acetone, modified if necessary by the mixture of other solvents into the solvent mixture. Similarly, the extraction of oily solids such as chopped nuts is best done with water-immiscible solvents such as benzene or chloroform (Tomsone, *et al.,* 2012).

Depending on if the analyte is part of a solid or liquid matrix, one distinguishes between solid-liquid and liquid-liquid extraction systems, respectively. For liquid-liquid extraction, solvent extraction involves the distribution of sample components between two immiscible liquid phases. If the extraction procedure is performed only once, then it is termed a single extraction method. Nevertheless, the extraction process may be repeated up to five times using fresh solvent each time to extract the majority of the solute from the solution. This is termed as multiple extractions. The separate extracts could then be combined if the concentration of the analyte in the final extract solution was too low. In addition, some of the solvent could be evaporated to reduce the volume in order to improve analyte concentration. The procedure of solvent extraction is simple, rapid and quantitative, requiring the minimum of apparatus (Tomsone, *et al.,* 2012).

The plant material used for extraction should be properly identified. The choice of the plant material for extraction depends on its nature and the components required to be isolated. The solvents used for extraction purposes is known as "Menstruum" and residue left after

extracting the desired constituents is known as "Marc". The active value of natural antioxidants of the plant is due to the active constituents (Tomsone, *et al.,* 2012).

SOLVENTS USED FOR THE EXTRACTION OF ANTIOXIDANTS:

When the material has extracted, the "Menstruum" is known as "Vehicle" or "Carrier" of the extracted materials. Solvents differ widely from each other, not only in differing boiling points, but how they act or react with substances in which they come in contact.

An Ideal Solvent for the extraction of the antioxidants should meet the following criteria:-
1. It should be non-toxic and selective, i.e. it should dissolve only the required constituent with minimum amount of the inert materials.
2. It should not cause the extract to complex or dissociate.
3. It should be preservative in action.
4. It should promote rapid physiologic absorption of the extract.
5. It should be easily evaporated at low heat.

Alcohol (Ethanol)

There are large number of solvents (Menstruum) used for extraction of antioxidants, but the selection of the suitable solvents capable of extracting the active constituents depends upon the chemical properties of active constituents as well as the qualities of the solvent. The solvents commonly used for the extraction of the antioxidants include water, alcohol and there different dilutions.

a. Water: - It is a good solvent for the extraction of many types of active constituents such as alkaloidal salts, colouring agents, glycosides, gums, sugars, anthraquinone derivatives and tannins. It can also act as menstruum for many organic acids and small proportions of volatile oils. Water is not a suitable menstruum (Solvent) for constituents like waxes, fats, fixed oil and alkaloidal bases due to their insolubility in water. Water is not selective as it can dissolve a wide range of substances and leads to hydrolysis of many substances. Water soluble herbs are aloe, glycyrrhiza, linseed, senna leaves, senna pods, ginger etc (Tomsone, *et al.,* 2012).

b. Alcohol: - Alcohol or ethanol can dissolve a large number of chemical constituents such as alkaloids, alkaloidal salts, glycosides, tannins, anthraquinone derivatives, volatile oils and resins, but constituents like albumin, gums, waxes, fats, fixed oils and sucrose are insoluble in alcohol. Generally dilute alcohols (hydroalcoholic solutions) are used for many extractions, but in some cases stronger alcohol may be used to prevent the extraction of unwanted substances such as gums. In a herb containing a number of chemical substances such as alkaloidal salts, glycosides, albumin and gum, water will dissolve all the substances, whereas dilute alcohol will dissolve only the alkaloidal salts and glycosides (Tomsone *et al.,* 2012).

c. Ether: - Soluble Constituents are oils, fats, waxes, resins and alkaloidal bases. Ether soluble herbs are capsicum, male fern, linseed, nutmeg etc (Tomsone *et al.,* 2012).

d. Chloroform: - Soluble constituents are oils, fats, waxes, resins and alkaloidal bases. It is non inflammable (Tomsone *et al.,* 2012).

e. Glycerin: - Soluble constituents are tannins. It is non inflammable and viscous liquid (Tomsone *et al.,* 2012).

f. Light Petroluem: - Soluble constituents are oils, fats, waxes, resins and alkaloidal bases. It is highly inflammable and very volatile (Tomsone *et al.,* 2012).

g. Fixed Oils: - Soluble constituents (Arachis Oil) can act as menstruum for camphor. It is non inflammable and viscous (Tomsone *et al.,* 2012).

h. Propylene Glycol: - Soluble constituents are progesterone, phenobarbitone sodium. Clear colorless, odourless, viscous liquid, miscible with water, alcohol and chloroform. Extraction of organic bases like alkaloids usually necessitates basification of plant material, if a water immiscible solvent is to be used, whereas for aromatic acids and phenols, acidification may be required. The glycosides are soluble in water and alcohol but are insoluble in non-polar solvents. Tannins are phenolic matter soluble in water, alcohol and ethyl acetate (Tomsone *et al.,* 2012).

FACTORS AFFECTING THE STABILITY OF EXTRACTS

Temperature and light are the major factors influencing antioxidant activity during storage. These factors affect different compounds to different extents. The reduction in antioxidant activity for ginger rhizome extract was more pronounced than those for potato peel and fenugreek seed extracts at each time of boiling indicates that antioxidant in potato peel and fenugreek seed extracts were fairly heat stable with 63.2 and 58% activity, respectively, still remaining after 120 min heating at 100 °C while for ginger rhizome extract was about 28% antioxidant activity after 120 min heating at 100 °C (Tomsone *et al.,* 2012).

The antioxidant activity was not affected by storage in dark conditions at 5, 25 and 37 °C over a period of 21 days while reduction was observed in light conditions with highest for potato peel extract (12.1%) followed by fenugreek extract (8.9%) and ginger extract (2.4%) extracts kept in light conditions at room temperature (25 °C).

The Holy basil and Galangal extracts showed strong antioxidant activity at neutral pH and weak activity at acidic pH possibly due to the different conformation and charges of antioxidant compounds under different pH values.

DIFFERNT ASSAYS TO ASSES ANTIOXIDANT CAPACITY OF PHENOLIC COMPOUNDS

It is necessary to have a convenient method to quantify the antioxidative effectiveness of an antioxidant. Total antioxidant power (content and effectiveness) is measured by a wide range of assays (Moon & Shibamoto, 2009). Based on the chemical reactions involved, there are two groups of assays to assess antioxidant capacity: Based on hydrogen atom transfer and another one are based on electron transfer. Hydrogen atom transfer based assays quantify the ability of an antioxidant to donate a hydrogen atom. Most hydrogen atom transfer based assays monitor competitive reaction kinetics, and the quantization is obtained from the kinetic curves. Electron transfer based assays measure the reducing capacity and often relies on a color change as readout (Moon & Shibamoto, 2009).

Folin-Ciocalteu assay

This is very popular, convenient, simple and reproducible, and is commonly known as the total phenolic compounds assay **(**Huang *et al*., 2005**).** A redox reaction occurs between the phenolic compounds and the Folin-Ciocalteu reagent under basic conditions (pH ~10) obtained using sodium carbonate. The reaction is monitored by the change in colour, which is proportional to the concentration of phenolic compounds. The reagent is non-specific, as it can also be reduced by other non-phenolic species (Stratil *et al*., 2006; Prior *et al*., 2005; Folin & Ciocalteu, 1927**).** However, the test has become a routine assay in studying phenolic compounds in plant materials and considerable amounts of data have been generated using the method **(**Huang *et al*., 2005**;** Moyer *et al*., 2002**).** Gallic acid equivalents (GAE) are used as reference in most cases **(**Magalhães *et al*., 2006; Vinson *et al*., 2001**).**

Radical Quencher assay

(Gokmen *et al*., 2009) reported the direct measurement of total antioxidant by using QUENCHER assay. Since the method described here is QUick, Easy, New, CHEap and Reproducible, it was called ''QUENCHER'' which sense immediately as a new approach for the measurement of antioxidant capacity. Since assays using 2,2′-azinobis-(3-ethylbenzothiazoline- 6-sulphonate) radical cation (ABTS) and 2,2′-diphenyl- 1-picrylhydrazyl radical (DPPH) are very popular and easy to use among the several methods described so far, the QUENCHER method was specifically exemplified for these widely established assays.

Oxygen Radical Aantioxidant Capacity (ORAC) assay

It is an example of a hydrogen atom transfer based assay in which an antioxidant and a substrate (fluorescein probe) compete kinetically for hydroxyl radicals. In this assay, as the reaction progresses, the antioxidant compound present limits the decrease in fluorescence, which is a meature of the extent of damage to the fluorescein probe. The inhibition of linoleic acid autoxidation by antioxidants is another example of a hydrogen atom transfer reaction.

Ferric reducing antioxidant power assay (FRAP)

This test uses a ferric complex Fe (III)(TPTZ)2Cl3 as an oxidant that is reduced to the ferrous Fe(II) form in contact with the antioxidant solution. The reaction is monitored for 4 to 6 min at 593 nm and the change in absorbance (ΔA=At- A0 min) related to ΔA of a Fe (II) standard solution, which is linearly proportional to the concentration of the antioxidant (Benzie and Strain, 1996). The drawbacks are associated with the specificity of the reaction because any compound with a redox potential lower than 0.77 V will reduce the ferric complex **(**Pérez-Jiménez *et al*., 2008; Huang *et al*., 2005).

1, 1-diphenyl-2-picrylhydrazyl radical scavenging capacity assay (DPPH• assay)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical widely used for evaluating natural antioxidants, algae or algal products due to its stability, simplicity and reproducibility (Balboa *et al*., 2013) The drawbacks are mainly due to the reactivity of the DPPH• radical, which may react slowly or not at all with some antioxidants, and possible

interference with compounds that present UV-Vis absorption maxima around 515 nm, leading to underestimations (Huang *et al*., 2005).

2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid radical cation decolourisationassay (ABTS•+)

The blue/green ABTS•+ is pre-generated through reaction between ABTS and potassium persulphate and diluted in ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. The reduction of the radical when mixed with antioxidants is monitored by spectrophotometric readings after 1 min and 6 min and results are expressed as Trolox equivalents (Re *et al*., 1999).

POTENTIALITY OF NATURAL ANTIOXIDANT

This study is to highlight the potentiality of different natural antioxidant which having the flavonoids, phenylpropanoids and phenolic acids, as important contributing factors to the antioxidant activity. Brewer M.S, 2011 reported that there are several mechanisms conferring by the natural antioxidant, to delay the lipid oxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical:

(1) By chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides.(2) By quenching $\cdot O_2^-$ preventing formation of peroxides.(3) By reducing localized O_2 concentrations (4) By breaking the autoxidative chain reaction.(5) By scavenging species which initiate the peroxidation(6) The transition metal-chelating potential.

CLASSIFICATION OF NATURAL ANTIOXIDANT

Based on their nature of action (Mukhopadhyay, 2006) categorised natural antioxidants in to five classes: 1) Primary antioxidants, substance which can terminate the free radical chain of lipid oxidation and function as electron donor for example tocopherol. 2) Secondary antioxidants, these can decompose the lipid hydroperoxides into stable end products. 3) Oxygen scavenger, compound which can react with oxygen and remove it into a closed system for example ascorbic acid. 4) Chelating agent, syngerstic substance which can enhance the action of phenolic antioxidant for example citric acid.

ANTIOXIDANT EXTRACTED FROM FRUITS, VEGETABLES, HERBS AND SEA FOOD

Ascorbic acid

Ascorbic acid also known as vitamin C which is a strong antioxidant found mainly in fresh fruits and vegetables (Kim *et al*., 2013). Depending on condition, ascorbic acid act as a chelating agent, a pro-oxidant, as an oxygen scavenger, a metal chelator or as a reducing agent. It inhibits oxidation at high concentration (1000 mg/kg) but in low concentration (100 mg/kg) it can catalyze oxidation (Ahn and Nam, 2004). Ascorbic acid has 4 −OH groups that can donate hydrogen to an oxidizing system. Because the −OH groups **are** on adjacent carbon atoms, Ascorbic acid is able to chelate metal ions (Fe^{++}). It also scavenges free radicals, quenches $\_O^{2-}$, and acts as a reducing agent. At high levels, Ascorbic acid shifts the balance between ferrous (Fe^{2+}) and ferric iron (Fe^{3+}), acts as an oxygen scavenger, and inhibits oxidation. However, at low levels (<100 mg/kg), it can catalyze oxidation (Yetella and Min, 2008). Environmental conditions and the presence of other compounds in the

system can alter the antioxidative capacity of Ascorbic acid.(Allam and Mohamed, 2002) reported that, using the induction period for the oxidation of sunflower oil as a measure of antioxidant activity after heating (180 °C), ascorbyl palmitate was less thermally stable than mixed tocopherols, propyl gallate, BHT, or BHA. This may be a function of the water solubility of Ascorbic acid (Ahn and Nam, 2004).

Citrus

Citrus forms an important source of bioactive compounds such as vitamin C flavonoids. The important flavonoids found in citrus are narirutin, hesperidine, naringin and eriocitrin. Flavonoid are abundant ilsewhere inthe plant kingdom, there are several compound such as flavanones, polymethoxylated flavones which are unique to citrus and these are generally rare in other plants. Ascorbic acid, a well known natural antioxidant, together with natural flavonoids are also attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Allam, 2004).

Clove

Eugenol, carvacrol, thymol, and cinnamaldehyde are commonly known as phenylpropanoids which is the primary component. It also contains various nonvolatile compounds such as sterols, tannins, flavonoids, and triterpenes. (Jirovetz *et al*., 2006) identified 23 compounds in clove oil including β- caryophyllene (17.4%), eugenol (76.8%), α-humulene (2.1%), and eugenyl acetate (1.2%). Clove essential oil has inhibitory action toward hydroxyl radicals, in addition with can chelate iron. Khatun *et al*., (2006) reported that clove had the the highest radical-scavenging activity among 16 different spices. (Politeo *et al*., 2010) observed that glycosidically bound volatile compounds in clove essential oil have significantly greater antioxidant activity than that of the volatile aglycones.

Cumin

Cumin is derived from Cuminum cyminum. The major components in cumin volatile oil are cuminal, γ -terpinene, and pinocarveol (El-Ghorab *et al*., 2010). Cumin essential oil is better at reducing Fe3+ ions than dried or fresh ginger or cumin.

Fish protein hydrolysate

Protein hydrolysates and isolated peptides prepared from marine blue mussel, hoki skin gelatine, Pacific hake fish fillet and tuna back bone has superior Antioxidative properties than α-tocopherol. In some cases it is equivalent to BHA, PG and BHT. Most of the recent studies reported that bioactive peptides from proteins act as alternative materials to synthetic antioxidants (Young Ko *et al.,* 2013) since protein hydrolysates produced from fish and fishery product for which no adverse or safety concerns have been identified. Use of these funtional ingredients in food products should not be limited by regulations such as those applying to BHA, BHT. Fish protein hydrolysates extracted from muscle generally contains 2-16 amino acids residues, low molecular weight, easy absorption, lower cost, and higher antioxidant activity (Sarmadi and Ismail, 2010). Some antioxidative peptides such as glutathione, carnosine, anserine and ophidine are naturally present in muscle tissues. All these bio-peptides have excellent antioxidant properties where Carnosine can act as a free radical scavenger as well as a metal ion chelator. On the other hand Glutathione acts as an

electron donor and its reducing power helps to maintain the reduced state of cysteinyl sulfhydryl groups in proteins that's why reduces disulfide bond formation within cytoplasmic proteins (Sarmadi and Ismail, 2010).

Antioxidative properties of fish proteins hydrolysates are related to their sequence, composition and hydrophobicity. Fish protein hydrolysate interacts with free radicals and terminates the radical chain reaction or prevents their formation by metal chelating or by electron donating. That's why the sequence of the peptides and constituents of the amino acid are very important for their antioxidant activity (You *et al.,* 2010). Several authors reported that the hydrophobic nature of amino acids and having more residues of Histidine, Proline, Methionine, Cysteine, Tyrosine, Tryptophan and Phenylalanine can enhance the activities of the antioxidant peptides. The presence of hydrophobic sequences in the peptides could interact with lipid molecules and could scavenge by donating protons to lipid derived radicals among hydrolysates, peptic hydrolysate having the highest antioxidant activity (Je *et al*., 2007).

The antioxidant activity of tyrosine is due to the special capability of phenolic groups to serve as hydrogen donors. The amino acids such as histidine (His), methionine (Met) and cysteine (Cys) are very important to the radical scavenging activity of peptides due to their special structural characteristics: the imidazole group in His has the proton-donation ability; Met is prone to oxidation of the Met sulfoxide; Cys donates the sulphur hydrogen. The exact mechanism of action of peptides as antioxidants has not clearly been understood, but some amino acids such as His, Met, Cys, Pro, Val, Phe, Tyr, Trp have been reported to contain vital role in the antioxidative activities of peptides or protein hydrolysates generated from fish proteins (Bougatef *et al*., 2010).

Garlic

Mostly two major classes of antioxidant compounds are found in garlic extract such as sulfur-containing compounds (allyl-cysteine, diallyl sulfide, and allyl trisulfide and flavonoids flavones and quercetins) (Brewer, 2011). These are mainly concentration dependant showing that garlic is an effective hydroxyl radical scavenger. Likewise garlic also contains ascorbic acid and nitrates and nitrites (Aguirrezabal, 2000). Garlic is rich in selenium and organosulphur compound, which have pronounced antioxidant activity (Yin *et al*., 2001)**. (**Amagase, 2006) demonstrated that thiosulfinates, such as allicin, give garlic its characteristic odor; however, they are not necessarily responsible for all of the various antioxidative and health benefits attributed to it. But the antioxidant activity of thiosulfinates in garlic extract require the combination of the allyl group ($-CH_2CH=CH_2$) and the –S (O) S− group (Okada *et al*., 2005).

Grape seed extract

Grape by-products contain significant amounts of phenolic compounds, mostly flavonoids. There are many references in the literature to the composition and antioxidant properties of grape polyphenols. It has also been reported that many of the anthocyanidins/anthocyanins, flavonoids and phenolic acids have antioxidant activities similar to synthetic antioxidants such as BHA and BHT (Sanchez-Alonso *et al*., 2007). Grape skins and seeds are rich source of these compounds including flavonoids with different degree of polymerization known as proanthocyanidins. The antioxidant activity of grape polyphenols mainly depends on the degree of polymerization. Oligomers (roughly 2–7

residues) are considered more efficient than monomeric counterparts due to their ability to concentrate where the oxidative reaction is likely to occur. Materials of higher degree of polymerization are also active but may be mucosal irritant and show astringency effects (Pazos *et al*., 2005). In addition to this resveratrol, quercetin, and rutin are also generally found in grape skin extracts. But in seed extract catechin and epicatechin are the primary component which can inhibit both lipid hydroperoxide and propanal formation in an emulsion system (Hu and Skibsted 2002). Including the skin, resveratrol is present in various parts of the grape. It has strong antioxidant activity exceeding that of vanillin, phenol, propyl gallate, BHT, and α-tocopherol this may be because it has more phenolic rings than propyl gallate, phenol, and BHT, and also have more −OH groups than α-tocopherol. Resveratrol inhibits peroxidation in a concentration-dependent manner (Murcia and Martinez- Tome 2001). Pazos *et al*., (2006) evaluated the effectiveness of a grape phenol fraction, isolated grape procyanidins, hydroxytyrosol (from olive oil), and propyl gallate in inhibiting lipid oxidation in a fish (hake) microsomal model system.

Marine algae

A huge number of new compounds isolated from marine sources have been increasing steadily (Blunt *et al*., 2012). Recently, there is a considerable interest in the food as well as pharmaceutical industry for the development of antioxidants from natural sources, such as marine flora and fauna as safe alternative of many synthetic commercial antioxidants. Among marine resources, marine algae represent one of the richest sources of natural antioxidants (Wijesekara, 2011). Among them, fucoidans derived from marine algae have a great antioxidants activity via their scavenging effect on biologically harmful oxidants. Fucoidan from the edible seaweed was shown to prevent the formation of hydroxyl radicals, superoxide radicals, and lipid peroxidation **(**Vo and Kim, 2012)**.** Natural pigments not only function as colorants, but also they contribute to the antioxidant activity of marine algae. It has been demonstrated that marine algae have potential antioxidant activity and various classes of natural pigments including fucoxanthin, phycoerythrobilin, chlorophyll a and their derivatives have been shown as potent antioxidants (Pangestuti *et al*., 2011) Antioxidant activity of natural pigments depends on their structural features such as porphyrin ring, phythyl chain and extended system of conjugated double bonds (Pangestuti *et al*., 2011). Fucoxanthin has a strong radical scavenging activity (Yan *et al*. (1999). Sachindra *et al*., 2007 arranged the order of scavenging activity of each carotenoid which is followed by: fucoxanthin > fucoxanthinol > halocynthiaxanthin. The potential involvement of fucoxanthin in radical scavenging activity may correlate to the presence of unusual double allenic bonds at C-7′ position. Therefore, fucoxanthin may have great potential for use as nutraceuticals and pharmaceuticals as a substitute for synthetic antioxidants. Two hydroxyl groups present in the ring structure of fucoxanthin may correlate to the inhibition of reactive oxygen species formation. Several studies have indicated that the number of hydroxyl groups on the ring structure of fucoxanthin is correlated with the effects of reactive oxygen species suppression.

Marjoram

Muchuweti *et al*., 2007 demonstrated that marjoram (*Origanum majorana L*.) has the highest proportion of simple phenolic compounds among the various number of spices and herbs.

Significant amount of rosmarinic acid and carnosol are available in marjoram essential oil. Edris *et al*., 2003 suggested that Marjoram essential oil is also rich in, p-cymene, terpinen-4-

ol, cis-sabinene hydrate and γ –terpinene. The essential oil can scavenge hydroxyl radicals (OH·). Schmidt *et al*., 2008 reported that 50% inhibition of the formation of initial compound during the oxidation of unsaturated fatty acids and 80% generation of secondary oxidation products by using marjoram essential oil.

Oregano

By comparing different herb and spices (Muchuweti *et al*. 2007) reported that oregano (Origanum vulgare L.) had the highest antioxidant activity. Primarily rosmarinic acid is the major phenolic component of oregano extract, which can prevent color deterioration (Hernandez-Hernandez *et al*., 2009). In addition to this oregano extract also contain phenolic carboxylic acids and glycosides that are both antioxidative and effective superoxide anion radical scavengers (Nakatani, 2003).

Potato peels

Potato peels are the major by-product of potato processing industries, which represent a major waste disposal problem. There is a great interest to up-grading of this by-product to value added products in the potato industries. Toma *et al*., 1979 reported that Potato peel extracts have good source of dietary fibre and Nielsen and Jacobsen, 2010 suggested it is also rich in phenolic acids especially of chlorogenic, gallic, protocatechuic and caffeic acids. Potato peel extract from Sava variety of potatoes were highly efficient in reducing lipid peroxidation both in fish oil and in oil-in-water emulsions (Habeebullah *et al*., 2010). However, the use of potato extracts as an antioxidant in fish muscle, which is a complex mixture of proteins, lipids, prooxidants such as haemoglobins, iron and antioxidants such as alpha tocopherols, ascorbic acid has not yet been studied (Farvin, 2012).

Rosemary

Rosemary (*Rosmarinus officinalis*) have multifunctional action, it can act as a chelating agent, scavenge superoxide radical and can inhibit the lipid oxidation. Peschel *et al*., 2007 reported total phenol content of rosemary is near about 150 mg/g. (Nakatani, 2003) reported the most active antioxidative constituents of rosemary are phenolic diterpenes (rosmadial,carnosic acid, rosmanol, carnosol, 12-methoxycarnosic acid, epi-, and iso-rosmanol) and phenolic acids (caffeic androsmarinic). (Richheimer *et al*., 1996) suggested that Carnosic acid has several times the antioxidative activity as BHT and BHA and more than twice that of any other phenolic compounds. A single aromatic ring with one –OH group is available in both BHA and BHT, which can able to donate the H·. But in case of carnosic acid, it has a single aromatic ring and 2 −OH groups which can serve as H· donor. (Bhale *et al*., 2007) informed the capabilities of rosemary extracts in retarding lipid oxidation of different fish oils. (Tironi *et al*., 2010) reported the ability of aqueous extracts of rosemary to prevent the b-carotene destruction in fresh and frozen muscles from different fish species.

Sage

Nakatani, 2003 reported that varity of antioxidant compounds from sage (*Salvia officinalis*) including rosemadiol, carnosol, epirosmanol, rosmanol, isorosmanol, carnosic acid and galdosol. (Papageorgiou *et al*., 2008) suggested that the antioxidative activity of sage oil correlates with the concentration of sesquiterpene and oxygenated diterpene, in addition with having –OH group. .

Seaweeds

(Farvin and Jacobsen 2013) reported a huge number of potent antioxidant compounds from different types of seaweeds, including sulphated polysaccharides, phlorotanins, carotenoid pigments such as fucoxanthin and astaxanthin, sterols, catechins and proteins. Three interconnected rings are found in terrestrial biophenols (flavonoids), while seaweed phlorotannins have up to eight interconnected rings, making them 10-100 times more powerful and more stable as free radical scavengers than other polyphenols. Antioxidant capacity and total phenol contents of seaweed mainly depend of species. Generally the green seaweeds (Caulerpa spp.) have higher free-radical scavenging properties, followed by the brown seaweed (Sargassum polycystum), then the red seaweeds (Eucheuma cottonii, Eucheuma spinosum and Halymenia durvillaei), or brown seaweeds (Dictyota dichotoma and Padina sp.) (Mahamed *et al*., 2012). A high correlation between the total phenolic content and antioxidant activity has been reported by many researchers. Seaweed polyphenols such as phlorotannins have been reported to scavenge free radicals, superoxide radicals, peroxyl radical, chelate ferrous ions and nitric oxide. Phlorotannins such as eckol, dieckol, phlorofucofuroeckol A and 8,8′-bieckol isolated from the Japanese brown algae *Eisenia bicyclis, Ecklonia cava* and *Ecklonia kurome* have shown 2–10 times more antioxidant activities as compared to catechin, α-tocopherol and ascorbic Acid. These compounds also showed a potent inhibition of phospholipid peroxidation in a liposome system (Gupta *et al*., 2011)

Spices and herbs

Spices and herbs both have significant antioxidative effects (Suhaj, 2006) due to having phenolic –OH group (Cai *et al*., 2005)**. (**Lugasi *et al*., 1995) suggested that plant having high levels of phenolic compounds such as gallic acid are effective scavengers of H_2O_2 and easily can donate hydrogen. Phenolic volatile oils are the principal active ingredients in most spices (Shan *et al*., 2005). Wojdyło *et al*., 2007 studied 32 different spices and identified major phenolic acids such as p-coumaric, neochlorogenic, caffeic and caffeic. Among the flavonoids apigenin, catechin, quercetin, kaempferol, naringenin and hesperetin are dominant

Tannic acids

(Maqsood *et al*., 2012) suggested that tannic acids or tannins are polyphenolic compounds commonly occurring in the barks, woods and fruits of many kinds of plants. Tannic acids exhibited the superior radical scavenging activities as well as reducing power and effectively inhibited the lipid oxidation in fish mince, fish oil-in-water emulsion and fish slices. Haemoglobin mediated lipid oxidation in washed Asian seabass mince was also impeded by incorporation of tannic acids. Generally it is considered as GRAS (Generally Recognised As Safe) by the Food and Drug Administration (FDA) at a level of 10-400 ppm for the use of an ingredient in some food product. (Maqsood and Benzakul, 2011). Maqsood *et al*., 2010 studied the four different phenolic compounds such as catechin, caffeic acid, tannic acid and ferulic acid. Among the four phenolic compounds, tannic acid was more effective in lowering the increase in PV in menhaden oil-in-water emulsion than the other three phenolic compounds. The higher efficiency of tannic acid in prevention of the hydroperoxide formation correlated well with the higher DPPH and ABTS radical scavenging activities and reducing power. Tannic acid contained a large number of

hydrophobic portions, which could align themselves at the oil–water interface and functioned as a hydrogen donor or radical scavenger. This resulted in the retardation of the initiation and propagation stages, as evidenced by the lower PV.

Green tea extracts

Among the basic three types of tea such as black, green and oolong, green tea has been acclaimed for its high antioxidant properties, attributed to the presence of highest total phenolics content and 94% of which are flavonoids. Oolong tea contains about 18% total phenolics and 4.4% flavonoids.Predominatly black tea contains thearubigins and theaflavins. Addition to this black tea also contains quinic acid, caffeic and chlorogenic (Brewer, 2011). Green tea is the major source of catechin and has been reported to have antioxidant, antimutagenic, antidiabetic, anti-inflammatory and antibacterial activities (Nirmal, 2011). Mostly the antioxidant activity of green tea is due to the presence of tea catechin including epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin (Tang, 2001). Abdullin *et al*., 2001 also suggested that the antioxidant activity of green tea extract due to presence of tannins, flavonoids and some vitamins. The antioxidant activity is linearly related to the phenol content. Sroka, 2003 sugested tea extract is the strongest antioxidant and H_2O_2-scavenging activity is due to phenols, with 3 −OH groups bonded to the aromatic ring, adjacent to each other. Epigallocatechin having highest antioxidant activity due to presence of 3 adjacent −OH substitutions on the B ring. Guo *et al*., 1996 described the Oder of antioxidative power in terms of free radical-scavenging ability, epicatechin gallate > epigallocatechin > epicatechin. Both epicatechin and epigallocatechin having trihydroxy group but epicatechin do not have. Green tea polyphenols are the most efficient inhibitors of mackerel muscle lipoxygenase (Banerjee., 2006) Liu & Pan, 2004 also reported the inhibition of gill lipoxygenase of tilapia and gray mullet by using green tea extract. Oxidative stability in ground white mackerel was improved by treated with green tea extract (He and Shahidi, 1997). Tea catechins were also reported to completely inhibit the pro-oxidant activity of all the skin extracts from selected fish (Mohri *et al*., 1999).

Thyme

Different species of Thyme contain 1,8-cineole which is having the antioxidative properties. (Miguel *et al*., 2004) reported that antioxidative activity of T. Caespititius comparable to that of vitamin E and BHT. Lee *et al*., (2005) informed about the different aroma compounds found in thyme, alphaterpineol, carvacrol, 1,8-cineole, thymol and linalool. Thyme essential oil inhibits lipid oxidation and also has strong free radical-scavenging ability (Bozin *et al*., 2006). In terms of antioxidative activity Youdim *et al*., 2002, arranged the different compound: thyme oil > thymol > carvacrol > γ-terpinene > myrcene > linalool > pcymene > limonene > 1, 8-cineole > α-pinene. Thymol and carvacrol each have 1 aromatic ring and 1 −OH group, p-cymene has 1 aromatic group while p-cymene has 1 aromatic group. The presence of aromatic groups and the number of −OH groups appears to coincide with the antioxidant potential of these compounds. (Brewer, 2011).

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4

Application of Edible Coatings and Films for Food Industry

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Coatings are applied and formed directly on the surface of the food product, whereas films are structures, which are applied after being formed separately (Guilbert, Gontard, and Gorris, 1996). Because they may be consumed, the material used for the preparation of edible films and coatings should be regarded as Generally Regarded as Safe (GRAS) (Park, Chinnan, and Shewfelt. 1994; Krochta and Mulder- Johnston, 1997) approved by Food and Drug Administration (FDA) and must conform to the regulations that apply to the food product concerned. The purpose of edible films or coatings is to inhibit migration of moisture, oxygen, carbon dioxide, or any other solute materials, serve as a carrier for food additives like antioxidants or antimicrobials and reduce the decay without affecting quality of the food.

Edible Coatings

Edible coatings (EC) are thin layers of edible material applied to the product surface in addition to or as a replacement for natural protective waxy coatings and provide a barrier to moisture, oxygen and solute movement for the food (McHugh and Senesi, 2000; Nisperos-Carriedo, Baldwin and Shaw, 1992; Lerdthanangkul and Krochta, 1996; Avena-Bustillos, Krochta and Saltvein, 1997; Guilbert *et al.,* 1996; Smith, Geeson and Stow, 1987). They are applied directly on the food surface by dipping, spraying or brushing to create a modified atmosphere (McHugh and Senesi, 2000; Krochta and Mulder-Johnston, 1997; Guilbert *et al.,* 1996). An ideal coating is defined as one that can extend storage life of fresh fruit without causing anaerobiosis and reduces decay without affecting the quality of the fruit (El Ghaouth, Arul, Ponnampalam and Boulet, 1992b). Previously, edible coatings have been used to reduce water loss, but recent developments of formulated edible coatings with a wider range of permeability characteristics has extended the potential for fresh produce application (Avena-Bustillos, Cisneros-Zevallos, Krochta and Saltveit, 1994). The effect of coatings on fruits and vegetables depends greatly on temperature, alkalinity, thickness and type of coating and the variety and condition of fruits (Park *et al.,* 1994). The functional characteristics required for the coating depend on the product matrix (low to high moisture content) and deterioration process to which the product is subjected (Guilbert *et al.,* 1996). Edible coating are also defined as thin layers of edible materials, but these are usually applied as a liquid of varying viscosity to the outer surface of the product by spraying, dipping, brushing or other appropriate methods. If desired, they could also be applied between layers of food components. To accomplish their intended functions, coatings are usually allowed to dry on the product after contacting the surface.

Figure1a. Coating on strawberry

Figure 1b. Coated Fresh cut Apples

Edible Films

Edible polymer film is defined as a thin layer of edible material formed on a product surface as a coating or placed (pre-formed) on or between food components (Krochta and Mulder-Johnston, 1997). Several types of edible films have been applied successfully for preservation of fresh products (Park *et al.,* 1994). Fruit based films provide enhanced nutrition for food products, while increasing their marketing allure (McHugh and Senesi, 2000). Edible and biodegradable films must meet a number of special functional requirements, for example, moisture barrier, solute or gas barrier, water/lipid solubility, color and appearance, mechanical and rheological characteristics, non-toxicity, etc. These properties depend on the type of material used, its formation and application (Guilbert *et al.,* 1996). The benefit of using selective films seems to be the reduction of water loss, which is one of the most important factors in the deterioration of highly perishables (Bussel and Kenigsberger, 1975). The films provide protection against moisture loss and maintain an attractive appearance of the product. Films may consist of single or multiple components (Guilbert *et al.,* 1996). Edible films are distinguished from coatings by their method of manufacture and application to the food product. Films are dried preformed thin material structures that are used on or between layers of food components. Edible films are usually between 50 to 250 µm in thickness and can be used to wrap the product or make pouches and bags. Several films can be combined to from laminated sheets.

Figure 2a. Edible film

Figure 2b. Food Materials packed in edible films

Specific requirements for edible films and coatings

1. It should be water-resistant so as to remain intact and to cover all parts of a product adequately when applied;
2. It should not deplete oxygen or build up excessive carbon dioxide. A minimum of 1-3% oxygen is required around a commodity to avoid a shift from aerobic to anaerobic respiration;
3. It should reduce water vapor permeability;
4. It should improve appearance, maintain structural integrity, improve mechanical handling properties, carry active agents (antioxidants, etc.,) and retain volatile flavor compounds.

Types of Edible Coatings and Films

Edible coatings may be composed of polysaccharides, proteins, lipids or a blend of these compounds (Li and Barth, 1998; Park *et al.,* 1994; Guilbert *et al.,* 1996; Mahmoud and Savello, 1992; Arvanitoyannis and Gorris, 1999). Their presence and abundance determine the barrier properties of material with regard to water vapour, oxygen, carbon dioxide and lipid transfer in food systems. However, none of the three constituents can provide the needed protection by themselves and so are usually used in combination for best results (Guilbert *et al.,* 1996). Some of the examples of edible coatings/films on fruits and vegetables are indicated in Table 1.

Table 1. Types of Edible Coatings and Films

S.No	Type of coating/film	Materials adopted	Commodity	Properties
1.	Polysaccharide based coatings/films	Starch ,pectin, cellulose, chitosan, alginate, chitosan, Hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC)	Apple, Lettuce, Litchi fruit, Kiwi fruit, avocado, Carrot, Mushroom, Citrus, Pears, water chestnut, Raspberry	• Excellent oxygen, aroma and oil barriers due to their tightly packed, ordered hydrogen bonded network structure and low solubility • Provide strength and structural integrity • Are not effective moisture barriers due to their hydrophilic nature • Retard ripening and increase shelf life of coated produce, without creating severe anaerobic conditions.
2.	Protein based coatings/films	Soy protein, whey protein, casein and corn-zein, maize, egg albumen, collagen and wheat	Celery, cherry, corn, carrot	• Excellent oxygen, aroma and oil barriers due to their tightly packed, ordered hydrogen bonded network structure and low solubility • Provide strength and structural integrity • The presence of several side residues of amino acids (cysteine) can inhibit polyphenoloxidase • Presence of fatty acids in whey protein also significantly improves moisture barrier properties • Proteins are good film formers and are produced from renewable resources and degrade more readily than other types of polymeric materials

3.	Lipid based coatings/films	Bee's wax, mineral oil, vegetable oil, surfactants, acetylated monoglycerides, carnauba wax and paraffin wax	Green bell pepper, mango fruit	• Use of milk protein based coatings could reduce enzymatic browning of cut fruits and vegetables • Limited oxygen barrier properties due to the presence of microscopic pores and elevated solubility and diffusivity • Lipid films have good water vapour barrier properties due to their low polarity, but are usually opaque and relatively inflexible • The three different forms of coatings mentioned above are not effective in preserving the quality of fruits and vegetables by themselves. • They are more effective when used in combination Example: • Plasticized protein films possess good mechanical properties and improve film systems • A film formed by milk protein (casein) and lipid (acetylated monoglyceride) provide protection from moisture loss and oxidative browning
4.	Composite coatings/films	Combination of the materials Polysaccharide+Protein+Lipid Polysaccharide+Protein Protein+lipid Polysaccharide+Lipid	Apples, Potatoes (Fresh cut), Mango fruit, Peach, Plum, Quince, Strawberry, Zucchini	

Method of application

The following are the methods generally adopted for the application of Edible coating/film on foods

1. **Dipping**

   The fruits/vegetables are dipped in the coating solution, strained and air dried and packed under suitable conditions.

2. **Spraying**

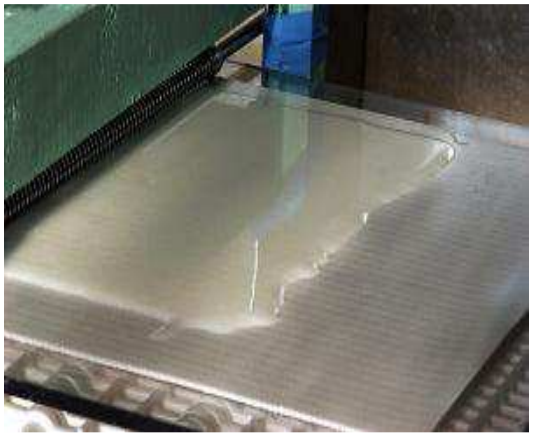
   The coating solution was sprayed over the fruits/vegetables and allowed to dry at ambient atmospheric conditions.

3. **Brushing**

   The coating material is applied on the food items using a brush with soft bristles and allowed to dry at ambient conditions

4. Casting

Edible films are usually produced by continuous film casting, mold casting or draw-down bar methods. The continuous film casting method is accomplished by coating a wet film onto a belt conveyor and then passing it through a drying chamber. Mold casting and draw down bar are simple and inexpensive methods that can be used as lab-scale edible film production techniques.



Advantages of Edible Coatings and Films

Advantages of edible coatings (Nisperos-Carriedo *et al.*, 1992; Park *et al.*, 1994; Sothornvit and Krochta, 2000) include:

1. Improved retention of color, acids, sugars, and flavor components
2. Reduced weight loss
3. Maintenance of quality during shipping and storage
4. Reduction of storage disorders
5. Improved consumer appeal
6. Stabilise the product and extend the shelf life
7. Addition of the value to the natural polymer material and reduction of synthetic packaging
8. Potential to reduce moisture loss and firmness loss
9. Provide moisture and oxygen barrier properties
10. Retard respiration rates
11. Hinder solute movement
12. Retard loss of chlorophyll

13. Retard ethylene production
14. Reduce metabolism and oxidation rates
15. Seal in flavor volatiles, carry additives that could reduce discoloration and microbial growth
16. Improve the appearance and very helpful in attaining relative humidity close to 100%
17. It can be consumed along with food, can provide additional nutrients, may enhance sensory characteristics and may include quality enhancing antimicrobials

Disadvantages of Edible Coatings and Films

While coatings have very desirable effects in reducing colour changes, firmness loss, and decay, there are some disadvantages. These disadvantages could be overcome by suitable selection of the type and thickness of the coating and by avoiding treatment of immature, flavorless fruit and storage of coated fruits at high temperature (Park *et al.,* 1994). However, since consumers are concerned with additives, including wax, acceptability of edible coatings must be recognized (Watada, Ko and Minott. 1996).

- Thick coatings could restrict the respiratory gas exchange, causing the product to accumulate high levels of ethanol and to develop off- flavors (El Ghaouth, Arul, Grenier and Asselin. 1992a; Howard and Dewi. 1995; Miller, Spalding and Risse. 1983; Davis and Hofmann, 1973).
- Films that have good gas barrier properties could cause anaerobic respiration and interferes with normal ripening (Meheriuk and Lau, 1988). The film should allow a certain amount of oxygen permeation through the coating or film in order to avoid anaerobic conditions.
- The spoilage could be rapid for coatings such as whey protein in moist environments, which serves as nutrient for microbial growth (Avena-Bustillos *et al.,*1997). Addition of antimicrobials like potassium sorbate to the coatings will be able to eliminate this problem.

CONCLUSION

Basic information on film-coating formulation, properties, methods of application to food surfaces and demonstration of effectiveness are lacking. Tremendous research is required in the area of applications of edible coatings of foods, especially fresh-cut fruits and vegetables.

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5

Carrot: Post Harvest Technology and Functional Aspects

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INTRODUCTION

Carrot is cultivated throughout the world and is considered one of the important root crops for its fleshy edible roots. It is grown in autumn, summer and spring in temperate countries and during winter in tropical and subtropical regions (Thompson and Kelly 1957). Carrots are used for human consumption as well as for forage particularly for feeding horses. They are cooked alone or with other vegetables in the preparation of soups, curries, pies etc.; fresh grated carrots are used in salads; tender roots are pickled, dehydrated and preserved in many other ways (FAO 1990; Kalra *et al*. 1987). The carrots are rich source of β-carotene and contains appreciable amount of thiamine and riboflavin and is said to possess many ayurvedic medicinal properties (Wealth of India 1952).

The carrots stand third in nourishing value in the list of roots and tubers, potatoes and parsnips are on first and second places respectively. Carrots contain less water and more nourishing material than green vegetables and therefore possess higher nutritive qualities than turnips, cabbage, cauliflower and onions. Moreover, the fair proportion of sugar contained in their composition adds to their nourishing value. Carrots contain more carotene than any other fruit or vegetable. They are bursting with Vitamin A which is good for bones, teeth, vision, and skin.

The carrots belong to the family *Umbelliferae* and to the genus *Daucus* and species *Carota*. The carrot (*Daucus carota* var. Sativa) is a native of Asia, Europe and Northern Africa along with some probable parts of North and South America. Many varieties of carrot are grown throughout the world and may be grouped into two types viz., (i) the temperate that are biennial (ii) the tropical that are annual, red in color, juicier, have a bigger core and a heavier top (Bose and Som 1986).

The important bunching varieties of carrot in the United Sates are Imperator, Gold Spike, Gold Pak, having long slender roots with a smooth exterior. The varieties, Royal Chantenay and Red Cored Chantenay, Autmn King are popular processing varieties, however, the highly flavored French or Chantenay varieties are not considered suitable for canned juice because of the development of the undesirable off flavors (Rodriguez et al. 1975; Kotecha et al. 1998). Carrot variety is reported to have a significant influence on the dry matter especially in terms of colour and sucrose content (Baardseth et al. 1995). The Royal Chantenay is about an inch longer and more cylindrical as compared to the Red Cored Chantenay. The other popular varieties are Perfection, Robicon, Hutchinson, Bunching, Bagley, Touchon and Coreless (Kotecha et al. 1998). Some important varieties of carrot that are commonly grown in various countries are: Amsterdam, Barlikuner, Chantenay, Royal Chantenay, Chantenay-Lysska. Delattya, Danvers, Emperador, Fertodi, Flakker, Gold Pak-28, Gonsen Lemer, Gross Lemer, Gross Karotel, Honey sweet, Karotena, Pusa Meghalli, Pusa

Kessar, Pusa Yamadagni, Berlicum, Kundulas, Nantes, Nanteska, Rubica, Rothild, Rote Rusen, Sperton Fancy, UH-AC, No. 29 etc.

In India, many cultivars, some indigenous but mostly introduced from Europe and America, are cultivated. Important among the exotic types grown in India are *Chantenay*, *Danvers*, *Nantes*, *Early Horn*, *Early Gem*. A crossed variety, named *Pusa Kesar* is the one cultivated mostly through out the India. This may be due to its good adoptability to the Indian climatic conditions with the most appreciated quality: high colour, desirable textural characteristic (firmness, tenderness or crispness, juiciness, etc), distinguished flavour for the commercial acceptability for the fresh market (Ghosh 2002).

Table 1 shows the carrot production worldwide. China has captured 34% production share followed by United States and USSR, each contributing 7%. Worldwide production of carrots has increased from 10.09 to 13.37 million metric tons (MT) during 1980–1990, a 30% increase over the past decade (FAO 1990). China is the leading producer in the World, the US ranks among the other top nations in the production of carrots: fourth in acreage and volume, third in terms of yield. The other major carrot producing countries are Russia, Japan, France and the United Kingdom.

Table 1: World carrot (*Daucus carota L.)* production in metric tons (1995 – 2005) (adapted from FAOSTAT DATA 2005)

Region	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
China	3,656,179	4,376,379	4,477,698	4,514,679	4,846,009	5,740,164	6,111,984	7,126,460	8,093,379	8,295,350	8,395,500
United states	1,878,000	2,043,000	2,266,950	2,128,300	1,941,950	1,858,610	1,837,440	1,537,220	1,637,700	1,601,790	1,601,790
Russian Federation	1,182,070	1,292,010	1,424,150	1,160,240	1,372,200	1,597,090	1,575,070	1,474,100	1,735,760	1,762,040	1730,000
Poland	814,311	794,128	799,428	991,955	906,477	946,736	921,911	692,073	834,621	927,949	935,000
Japan	724,700	736,200	716,100	648,100	676,700	681,700	691,300	643,700	658,700	613,400	630,000
United kingdom	517,200	617,400	623,100	617,600	673,200	725,800	760,000	731,200	617,800	619,300	677,140
France	637,851	644,060	652,000	662,000	672,000	658,376	649,489	727,645	688,426	703,218	417,800
Italy	396,443	346,185	457,829	471,806	509,849	642,065	590,997	561,442	571,160	607,188	641,558
Ukraine	408,500	318,600	449,000	379,700	384,700	496,500	462,500	445,400	529,700	674,900	706,500
Netherlands	368,900	350,000	350,000	277,000	448,000	385,000	378,000	422,000	432,000	430,000	430,000
Germany	297,411	340,082	312,723	371,950	379,544	431,541	444,448	414,960	426,038	554,000	555,000
India	300,000	320,000	340,000	340,000	340,000	350,000	350,000	350,000	350,000	350,000	350,000
Spain	304,592	301,541	356,695	333,250	400,562	425,357	383,311	436,099	448,349		
Mexico	199,588	219,500	306,753	319,926	363,368	376,847	355,903	378,517	378,517	378,517	378,517
Indonesia	247,179	269,837	227,322	332,846	286,536	326,693	300,648	282,248	355,802	423,722	308,675
Canada	288,710	320,400	290,510	324,842	294,183	261,284	279,050	286,496	313,344	293,810	301,450
Australia	238,539	249,926	257,405	266,531	256,608	283,304	320,908	331,129	305,669	302,560	302,560
Morocco	223,525	224,500	221,400	-	220,850	-	-	-	332,210	311,090	300,000
Turkey	250,000	270,000	240,000	232,000	239,000	235,000	230,000	235,000	405,000	438,000	380,000
Argentina	205,000	220,000	254,542	241,166	-	221,791	282,543	250,000	-	-	-
Belgium	-	-	-	-	-	-	-	-	-	292,250	320,000
Belarus	-	-	-	-	-	-	-	-	310,000	310,000	306,000
Nigeria	-	-	-	-	231,000	231,000	231,000	235,000	-	-	-
Venezuela,Bolivar	-	-	-	237,628	-	-	-	-	-	-	-

Annual production for the period 1995 – 2005 (adapted from FAOSTAT data 2005)
- not covered in that particular year under the top twenty countries

CULTIVATION AND HARVESTING

Carrots are a cool seasonal crop therefore mainly grown in the northern India during the winter. In the hills, the carrots are grown nearly throughout the year. The color varies from white to yellow, light purple, orange, deep red or violet and the shape varies from short stumps to tapering cones. The shape of the carrots makes them easy to handle during harvesting, cleaning, shipping and distribution. Their effective root length at harvest is 10 cm and the ratio of root length to maximum diameter is 25.0. The carrots contain 5 and 20 mg/100 g fresh weight lycopene and total carotenes, respectively (Kishida *et al*., 2007). The root diameter and length can vary in between 2-6 cm and 6-30 cm respectively.

Carrots are harvested when the roots are about 1.9cm or larger in diameter at the upper end. The soil may be loosened with a special plough (carrot lifter) or an ordinary plough. The field is irrigated one day before harvesting to facilitate the manual pulling. The carrot tops can be cut mechanically (Ryall and Lipton 1972) but the damage is substantial. The visible damage on roots to the extent of 20-30% has been reported during the mechanical harvesting (Kalra et al. 1987). Machine harvesting does not significantly affect chemical or sensory variables compared with hand harvesting (Seljasen *et al*. 2007).

The growth of carrot roots and its composition may depend upon the soil and climate, propagating conditions, and the cultural practices (sowing, manuring and fertilization, and irrigation and intercultural operations). The yield, root weight, quality grade, and carotene content increase and incidence of misshapen roots decreases with increasing level of addition of organic matter to the soil, irrespective of harvest date (Parraga *et al*., 1995). N-fertilization has been reported to increase in phenolic compound concentrations in comparison with the control (Smoleń and Sady 2009).

POST-HARVEST TECHNOLOGY

Handling and Storage

Handling

Freshly harvested carrots shall be sorted out to remove defective roots. Careful handling is necessary to avoid bruising and tip breakage during the grading steps. The carrots can be sold as topped or bunched carrots (tops left on) depending upon the market requirements. Bunched carrots deteriorate faster, than topped carrots. Additional post-harvest shaking can cause a genetically-variable increase in respiration rate and ethylene production, as well as increased ethanol and 6-methoxymellein generation and reduced levels of various terpenes and sugars. Shaking tended to increase bitter taste, earthy flavour and after taste, as well as terpene flavour and aroma. Bolero, Panter and Yukon varieties were found most susceptible to mechanical stress, therefore careful handling of carrots during distribution is important in sensory terms (Seljasen *et al*., 2007).

After harvesting, the carrot roots are topped, washed, graded and packed for fresh marketing or storage. The size grading is generally carried out by divergent rollers or belts (Burns 1997). Various sizes can be sorted out, including the removal of very large or small roots. The roots in the range of 2.5 to 3.5 cm in diameter are selected for canning. Topping and bagging greatly reduces the loss of moisture during transportation to the market in

addition to enhancing the shelf life of carrots. The packaging films are perforated to prevent the development of off-flavors.

Preconditioning and pre-cooling

The rapid lowering of temperature of harvested produce nearby storage temperature is termed as pre-cooling, which produces greatly increased storage life. The most common pre-cooling methods are: room cooling, forced air cooling, hydrocooling, package icing, vacuum cooling and evaporative cooling. Package icing and room cooling methods are generally considered for the pre-cooling of carrots. However, hydrocooling is also considered a method of pre-cooling for medium to large produce operations. High humidity is necessary to prevent dehydration and loss of crunchiness of stored carrots. Prompt cooling to 5^0C or below after harvest extends the shelf life of carrots during storage (Hasselbring 1927). Toivonen *et al*., (1993) observed that the four days preconditioning at 1^0C was found to significantly reduce weight loss of carrots.

Carrots stored below 0°C for prolonged periods of time are susceptible to chilling injury. The precaution should be taken to avoid, mixing carrots with ethylene-producing commodities such as apples, peppers or melons etc. during storage. Blanching carrot slices, particularly with 0.21% citric acid, before drying is reported to enhance inactivation of *Salmonella* during home-type dehydration and storage (Patricia *et al*., 2007).

Pre-packaging

The suitability of different packaging materials for the pre-packaging of carrot produce has been studied for long term storage of carrots. Umiecka (1981) carried out the experiments on the influence of variety, plant density, sowing and harvesting time on the suitability, and also the keeping quality of carrots for prepackaging in different means of polyethylene bags and storing conditions. In favorable conditions, the different varieties with equal storage ability were kept for 8-9 months. The carrots packed in either perforated polyethylene bags or in crates lined with polyethylene films could be stored for 8-9 months at 0-1°C (Umiecka 1981). The losses of biologically active components during storage of carrots are lower in polyethylene bags. Controlled atmosphere storage of sealed polyethylene bags of carrots was found very effective (Phan 1974).

Quality Criterion of fresh carrots

The quality characteristics of fresh carrots depend on various factors. Color plays an important role and the produce is acceptable on account of the attractive appearance. Measurement of Hunter "a" value using a colorimeter has been reported a simple and reliable method to evaluate carrot quality (Park-Se-Won *et al*., 1995).

Many countries have framed standards for the selection of carrots. The Bureau of Indian Standards(BIS) have suggested three grades 'Super', 'Fancy' and 'Commercial'. Carrots falling under Super grade should be of similar varietal characteristics, fresh and not withered or wilted, tender, well shaped, smooth, firm, reasonably clean, well trimmed and uniformly colored. The carrots should be free from seed stalk, growth cracks and sun burns, free from damage caused by disease, insect, mechanical or by other means. The carrots falling under Fancy grade shall have similar characteristics as super grade, but are slightly

inferior in quality. The carrots falling under commercial grade do not confirm to the requirements of either Super or Fancy grade but the quality is fit for use of human consumption.

Storage

Carrot roots are considered to be highly susceptible to moisture loss leading to wilting, limping and loss of fresh appeal. Topped carrots may be stored for 5-6 months without any loss in quality in dry sand, sometimes mixed with wood ash and charcoal (Wealth of India 1952). It is reported that storing carrots in pits, packages or stores with active ventilation results in wilting and deterioration. The degree and speed of changes depend on the temperature and relative humidity (RH) of the environment. Henze (1974) studied about the short-time-storage of carrots and processing by drying and found that depending upon the variety and degree of damage, lower graded carrots in terms of quality can be stored up to 2 months without higher losses. Various methodologies as discussed below are adopted for the storage purposes.

Hypobaric Storage

The storage involves the cold storage of produce under partial vacuum. Typical conditions include pressures as low as 80 and 40 millimetres of mercury and temperatures of 5°C (40°F). Hypobaric conditions reduce ethylene production and respiration rates; the result is an extraordinarily high-quality produce even after months of storage (http://www.britannica.com/EBchecked/topic/279852/hypobaric-storage).

The use of low pressure storage (LPS) at 8 kPa and near-saturation relative humidities (and in some cases, low relative humidity) for selected vegetable crops over various storage periods and temperatures is now gaining importance in extending the shelf life. Hypobaric storage containers/chambers work by maintaining 10 to 15 Torr vacuum regime with restricted airflow providing 0.13 to 0.2% oxygen, 0% carbon dioxide, no ethylene, and nearly 100% relative humidity at optimal constant temperature. Under these conditions, production and retention of the ripening hormone, ethylene and commodity respiration are drastically reduced, thus preventing bacterial and fungal decay, ripening, wilting, and other causes of deterioration. This process is completely chemical free with nothing added and environmental sustainable. This condition also kills insect eggs, larvae, and adults hitchhiking on fruit and vegetables (www.atlasuhv.com).

Bangerth (1974) reported that storage of some vegetables and vegetable fruits at hypobaric storage conditions to extend their storage life. McKeown and Lougheed (1981) also investigated the potential of use of low pressure storage system (LPSS) for the carrots. LPS provides a simple means for reducing the effect of ethylene-producing vegetables upon carrots, when stored together, but with no ethylene source in the storage environment there seems to be little benefit of LPSS. The use of LPSS is not wide spread due to the higher application cost and expensive in nature.

Low temperature storage

The temperature and Relative humidity control play an important role in the extension of shelf life of fresh fruits and vegetables. Weight losses in carrots during storage are mainly caused by transpiration. Respiration losses are small at low temperatures and at higher

temperatures where respiration is high, carrots can be stored only for short periods (Apeland and Baugerod 1971). Mature topped carrots can be stored for 7 to 9 months at 0 to 1°C with a very high relative humidity, 98 to 100 percent. However, even under these optimum conditions 10 to 20 percent of the carrots may show some decay after 7 months. Prompt cooling to 5°C or below after harvest is essential for extended storage. The deterioration in quality during the storage is due to the slow loss of sugars in respiration. The loss can be minimized under the good storage conditions.

Apeland and Hoftun (1974) suggested that for long term storage of carrot it should be stored between 0 - 1°C. The carrots intended for storage should be cooled to storage temperature (0°C) as quickly as possible after harvest and store at as high a level of relative humidity as possible. When relative humidity is lowered than desired, the roots looses substantial amount of water (6-8%). These roots may be rehydrated in ice water where they regain moisture at the rate of 2.6% per day. Pantastico et al. (1975) recommended a storage temperature of 0°C for topped or bunched carrots. The storage life of topped carrots is considerably longer (about 20-24 weeks) as compared to the bunched carrots (4 weeks), which require slightly higher relative humidity as compared to the topped carrots. Carrots packed in either perforated polyethylene bags or in crates lined with polyethylene films may be stored for 8-9 months at 0 – 1°C (Umiecka, 1981).

The optimum storage temperature for carrots as recommended by International Organization for Standards (ISO) is 0 – 1°C with RH 95-98%. Maximum recommended storage height for carrots stored in bulk is 1.75m, and for bags, 3m. Air circulation should be particularly vigorous for bulk-stored carrots at heights approaching maximum values (ISO 1974). Root hexose content increases rapidly, and sucrose content decreases during the first 60-90 days of 6 months storage, at 0-1°C/95-98% RH after which there was little further change (Nilsson 1987).

Modified and controlled atmosphere storage

Modified atmosphere storage (MAP) is the replacement of air (N_2, 78%: O_2 21%; CO_2 0.035% together with water vapor and traces of inert gases) in a pack with a single gas or mixture of gases; the proportion of each component is fixed when the mixture is introduced. No further control is exerted over the initial composition whereas the controlled atmosphere (CA) technique requires constant monitoring and control of the gas composition within the package or storage facilities and is used primarily for the bulk storage and transport of products.

The successful application of controlled atmosphere storage conditions in fruits and vegetables is based on the inhibition of the respiratory metabolism. But this method is not practicable in those cases where the respiratory inhibition leads to the qualitative changes of the metabolism with the formation of compounds showing adverse affects on the tissue. The critical concentration of CO_2 and O_2 in the storage atmosphere for the various kinds of fruit and vegetables are defined by the storage tests. Hansen and Rumpf (1974) reported that the production of isocoumarins in carrots which, when present, may impart a bitter flavor, may be inhibited in an atmosphere of 1.5 – 3% O_2. Van den Berg and Lentz (1966) reported that storage in modified atmosphere (3-6% O_2 with 0.5, 5 or 10% CO_2) generally increases the mold growth. Modified atmosphere (MA) packaging retards whitening of carrots but had a detrimental effect on firmness (Lafortune 2005). Bohling and Hansen (1981) found that

controlled atmosphere (high CO_2 and low O_2) is not beneficial for carrots since this leads to the growth of rootlets and shoots on carrots.

Stoll (1974) reported that in controlled atmosphere storage tests the different morphological vegetable structure does not show similar rates of conservation. Baumann (1974) studied the effect of low temperature storage and controlled atmosphere storage for 5–6 months on the quality of two different varieties of carrots. Low oxygen level upto 3% favor the preservation of carotene and total sugar. Carbon-dioxide level of 1% proves to be more advantageous than higher ones. The percentage of sugar is not altered by the cold store but is altered by storage in controlled atmosphere without scrubbing (5% CO_2 + 16%O_2).

The physiology and quality of carrot (*Daucus carota* L.) slices, sticks and shreds stored in air or controlled atmosphere (CA) of 0.5% O_2 and 10% CO_2 at different temperatures (0 - 10°C) indicated that the CA reduced the respiration rate of carrot slices, sticks and shreds at all temperatures, and have desirable effects on storage quality. Carrot sticks and shreds benefitted from CA storage at 0 and 5°C but not at 10°C. The study concluded that at 0°C, cut carrots can be held in film bags with O_2 levels of 0.5% and CO_2 levels of 10% without deleterious effect (Izumi *et al*., 1996).

The changes in nitrate and nitrite content were studied during controlled atmosphere (CA) storage (0% CO_2+ 1% O_2) at 0-1°C. No clear relationship is shown between nitrate-nitrite conversion and enzyme activity during storage and between different CA conditions (Yang-Yong-Joon and Jeong-Jin-Choel 1993). The results suggest that conversion of nitrate to nitrite might be by anaerobic microbial action, not by action of endogenous nitrate reductase during storage. Gomez-Lopez *et al*., (2007) explored the possibility of using of gaseous chlorine dioxide as a promising alternative to prolong the shelf-life of grated carrots.

Changes during storage

Physico-Chemical

During storage (temperature of 1^0C and RH of 98%), total sugars remains almost constant whereas the ratio of non-reducing sugars to reducing sugars begins to decrease and then remains steady. After 8 weeks of storage it increases again, reaches a maximum between 14 and 18 weeks and then decreases which may be related to the synthesis of oligosaccharides induced by spring reactivation. Phenol content increases with time; moreover, when ethylene is present, it induces the formation of isocoumarin and eugenin as well as two other related compounds (Phan *et al*., 1973). The sweetness increased during the storage with air atmosphere but reduced in presence of ethylene. Harsh flavor did not change when stored in air but changes may arise with bitterness in carrots exposed to ethylene (Simon 1984).

Moisture, protopectin and hemicellulose contents are reported to decrease and fibrous matter and pectin to increase in carrots during storage. The effect of storage on the quality of carrots has been studied and found that carotene concentration decreases and peroxidase activity increases. The quality of carrots deteriorates during storage owing to slow loss of sugar in respiration.

Carrot (*Daucus carota* L.) roots stored at 3±1^0C in an atmosphere containing 100 µl/l of ethylene had their total phenol content increased markedly as compared to control samples

kept in air. Isochlorogenic acid and eugenin are synthesized along with the isocoumarin during the development of bitterness in carrot (Sarkar and Phan 1979). Ethylene concentration and temperature is reported to increase respiration and favored a more rapid formation of isocoumarin (Lafuente 1996).

Harvested carrots accumulate an antifreezing protein in their cell walls reaching a maximum level after 12 weeks of storage at 0°C, followed by a gradual decrease. The appearance and accumulation of the antifreezing protein suggest that structural changes leading to changes in mechanical properties during the first 12 weeks of storage might be associated with a cold acclimation process (Gomez *et al*., 2003).

Diseases and Remedies

The diseases associated with the storage of carrots involve gray mould rot (*Botrytis cinerea*), watery soft rot (Sclerotinia sclerotiorum), black rot (*Stemphylium radicinum*), bacterial soft rot (*Erwinia carotovora*) and fusarium soft rot (*Fusarium*) (Frazier and Westhoff 1978).

Temperature and relative humidity both enhance the bio-deterioration of the carrot root through an increase in the diameter the rotted area of infected roots. The rot during storage was caused by *Rhizopus oryzae, Trichoderma harzianum, Botryodiplodia theobromae, Aspergillus niger* and *Aspergillus flavus*. The amounts of total soluble sugar in rotted carrot root were substantially decreased 2 – 4 days after infection. The presence of glucose, maltose, sucrose, lactose and galactose was evident in healthy carrot roots, while only lactose and galactose were present in infected roots. The ascorbic acid, total nitrogen, crude protein, crude fibre, fat and mineral contents in infected carrot roots reduced as the storage period was prolonged (Odebode and Unachukwu 1997).

In gray mould rot disease, the whole root may be affected, though the lesion usually occurs at the crown or at the tips or at injuries (Ryall and Lipton 1972). This disease is frequent during storage and is caused by air borne spores and spread by root to root through contact. In black rot, the infection usually occurs during storage at the crown and penetrates into the core. Black mold may occur on the surface and the affected tissues are soft and wet. The most preventive measure is to remove the visible infected roots before storage. The diseases may be controlled by careful handling of roots and storing them at 32^{o}F with RH 95%.

In watery soft rot, the infection is largely through wounds and occurs any where on stored carrots. *Sclerotinia* rot is the most prevalent and infects the carrot roots in the field through the crown. *Sclerotinia* fungus produces a white, cottony mycelium during storage, which covers the roots. Storage losses to *Sclerotinia* can be minimized by a) pre-harvest application of fungicide, b) rapid removal of field heat from freshly harvested roots c) increasing row width to increase ventilation since prolonged periods of leaf wetness induce rot d) crop rotation and e) post-harvest application of antimicrobials. Carrot cultivars grown were evaluated for susceptibility to the fungal leaf blight pathogens *Alternaria dauci* (1999) and *Cercospora carotae* (2000–03) in an experimental field under continuous carrot cultivation since 1996. Of the cultivars evaluated, Bolero, Carson, Calgary, Ithaca, and Fullback were less susceptible to *A. dauci* whereas, Bolero, Carson, and Bergen were less susceptible to *C. carotae*. Fontana was most susceptible to both fungal leaf blight pathogens (Gugino *et al*., 2007).

Carrots treated with the chemicals, like thiabendazole, quintozene, formaldehyde etc. before storage exhibit strong resistant to rots, caused by various microorganisms during storage. Dichloran has been reported to be very effective in controlling *Rhizopus* and *Monilinia* infections (Eckert and Ogawa 1988; Eckert 1977). Greenhouse-grown carrot plants, sprayed with an extract (0.2%) of the seaweed *Ascophyllum nodosum* (SW) enhanced disease resistance against fungal pathogens *Alternaria radicina* and *Botrytis cinerea.* The disease resistance in carrot may be due to the induction of defence genes or proteins (Jayaraj *et al*., 2008).

Fresh carrots treated with or without a continuous atmosphere of 50 ± 10 nL L^{-1} ozone during storage for up to 6 months at 0.5°C and more than 95% relative humidity showed reduced lesion size and rate of expansion against *B. cinerea*e. The incidence of carrots harboring visible saprophytic mold on the crown was substantially reduced in the ozone treatment. Ozone-induced injury, appearing as blotches of brownish discolored periderm, was slight, but increased with time whereas carrots in the control treatment did not become discolored. No effect of Ozone treatment on fresh weight loss, sprouting of carrot crowns, glucose, sucrose, fructose, or galactose concentration was observed. Levels of isocoumarin were slightly higher and averaged 17.8 mg kg^{-1} compared with 12.1 mg kg^{-1} in the controls and may have been associated with reduced lesion growth by *B. cinerea*. The ozone treatment can be useful for reducing nesting caused by *S. sclerotiorum* and *B. cinerea* in carrots, meant for the processing (Hildebrand *et al*., 2008). The chitosan treatment to the carrots induces host resistance to the *Sclerotinia sclerotiorum* during storage (Molloy *et al*., 2004).

CARROT: FUNCTONAL ASPECTS

Carrot is termed as functional food; as its intake in natural, conventional or processed form imparts health benefits in addition to its basic nutritive value. Carrot is rich source of various key classes of functional food ingredients such as vitamins, phyto-chemicals, phyto-nutrients, potassium, magnesium, manganese etc (Kaur and Sharma, 2013; Kaur and Sharma, 2011). Carrot being enriched with various advantageous natural biologically active ingredients, is placed in the category of Basic Functional Food i.e. food product with naturally occurring functional properties because of presence of natural functional ingredients. The presence of these bioactive functional ingredients has active physiological effect and perhaps enhances health benefits by imparting disease preventing as well as health promoting function. Carrot is credited with many medicinal properties of anti-carcinogenic, anti-inflammatory, antiaging, remineralizing etc. and is receiving much attention by food and nutrition scientists because of its high nourishing value. The compositional pattern of the raw carrot is given in Table 2.

Table 2. Composition of Raw Carrots

Particulars	DFCDB(2009)
Moisture (%)	89.5
Protein (%)	0.7
Fat (%)	0.4
Carbohydrates (%)	8.8
Crude Fiber (%)	-
Total Ash (%)	0.7
Calcium (mg/100 g)	25
Iron (mg/100 g)	0.24
Phosphorus (mg/100 g)	33
Sodium (mg/100 g)	66
Potassium (mg/100 g)	286
Magnesium (mg/100 g)	10
Copper (mg/100 g)	0.04
Zinc (mg/100 g)	0.19
Carotenes	1,890 (µg/100 g)*
Thiamine (mg/100 g)	0.04
Riboflavin (mg/100 g)	0.033
Niacin (mg/100 g)	1.0
Vitamin C (mg/100 g)	7.0
Energy value	150 (kJ/100 g)

Source: * Goplan *et al*., (1989)

: Danish Food Composition Data Bank (DFCDB) (2009)

With the increasing awareness, the demand for nourished and functional food is increasing. Therefore, research towards the development of functional foods is the area getting wider attention. Utilization of a carotene-rich functional food ingredient recovered through mechanical and enzymatic breakdown of the tissue of carrot pomace has also received a considerable attention by the researchers (Stoll *et al*., 2003: Upadhyay *et al*., 2008: Upadhyay *et al*., 2009; Navneet *et al*., 2012).

Carrot is rich source of antioxidants, vitamins and dietary fiber. The consumption of natural antioxidants is the prime concern of the consumers. Antioxidants are substances, which inhibit or neutralize the harmful effects of free radicals and help in repairing cellular damage resulting from oxidation. These work by binding with the free radical and providing the extra electron required to make a pair. Antioxidants stop chain reaction of free radical formation. Since antioxidants are used up in the process of neutralizing free radicals therefore it, is important to maintain a regular equilibrium between free radical formation and antioxidant intake.

Bioactive substances:

Carrot is a good source of number of bioactive substances therefore has anti-inflammatory, anti-cancerous, antiseptic, anti-bacterial and anti-aging properties. Carotene being antioxidant prevent degenerative processes in the cells and has great anti-aging effects. Carrots are especially rich source of β-carotene which is precursor of vitamin A, which exhibits strong antioxidant properties. Carrots are categorized on the basis of its colour pigment. The carrots in different category differ in their bioactive substances. Red and purple carrots are rich in rich in anthocyanin content whereas orange carrots are rich in beta-carotene, which accounts for 65% of their total carotenoid content. In yellow carrots, lutein is

in major amount. Beta-carotene is the major carotene and one of the powerful natural anti-oxidant which helps protect body from harmful oxygen-free radical injury.

Carotenes are converted into vitamin A in the liver which has various beneficial functions in human body such as improvement of eywsight, maintenance of epithelial integrity, growth and development of body. Recent research explored another category of phyto-nutrients in carrots called polyacetylenes. The important polyacetylenes in carrots include falcarinol and falcarindiol. The carrot polyacetylenes as phytonutrients can help to inhibit the growth of colon cancer cells. Preliminary research on animals and in the lab has shown that carrot polyacetylenes have anti-inflammatory properties and anti-aggregatory properties (that help prevent excessive clumping together of red blood cells)
(http://www.whfoods.com/genpage.php?pfriendly=1&tname=foodspice&dbid=21).

Carrot contains some dietary fiber, which helps in lowering cholesterol, control sugar level and help protect bowel cells from cancer causing damage. Vitamin C which is water soluble anti-oxidant, helps in maintain collagen, healthy connective tissue, teeth and gum, as well as protect from diseases and cancers by scavenging harmful free radicals.

The appreciable amount of B-complex groups of vitamins in carrots, act as co-factors to enzymes during substrate metabolism in the body (e.g. folic acid, pyridoxine, thiamin, pantothenic acid, etc.) The good amount of vitamin K in carrot helps in the formation of new bone material and healthy bone structure and to coagulate blood. Carrots are composed of high levels of minerals like manganese, magnesium and potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure by countering effects of sodium leading to anti-cardio vascular disease. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase (http://www.nutrition-and-you.com/carrots.html). Carrot extract have found to have inhibitory effect on mutagenicity of *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodibutylamine (NDBA), and *N*-nitrosopiperidine (NPIP). Carrot extract was reported to have highest inhibition effect on NDBA and NPIP (Ikken *et al.*, 1999)

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6

Food Proteomics

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INTRODUCTION

Proteins are type of organic compound, large and complex collections of molecules. They are the major molecules from which living things are constructed. Proteins are composed of units called amino acids. Amino acids contain the elements of carbon, hydrogen, oxygen and nitrogen. Certain amino acids also have sulfur atoms, phosphorous, or other trace elements such as iron or copper.

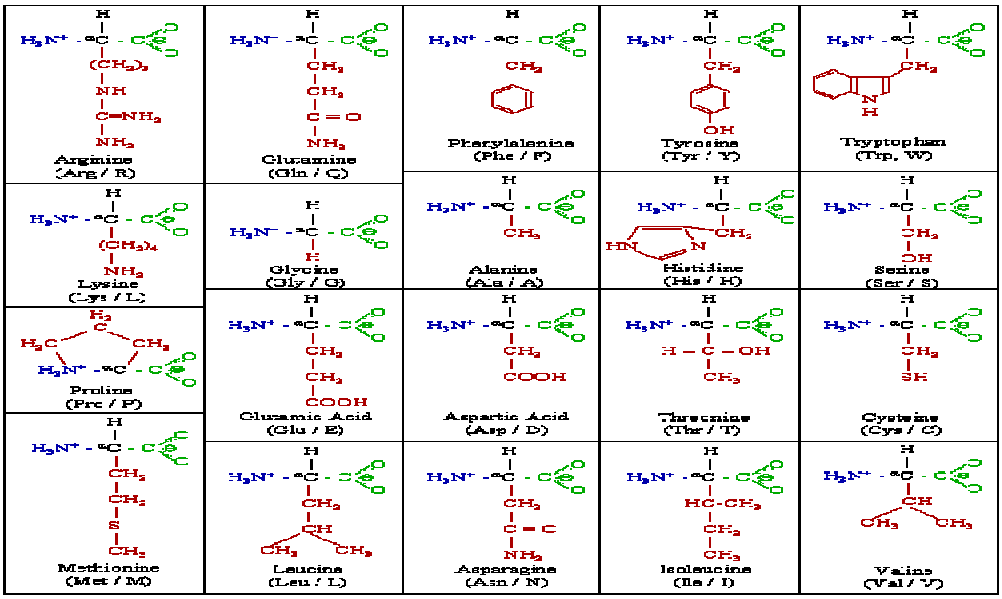


Fig. 1: **Twenty standard amino acids**

In proteins, amino acids are linked together like units on a chain. There are 20 different amino acids (Fig.1). Each amino acid contains a central carbon bonded to four main groups: an amino group ($-NH3^+$), a carboxyl group ($-COO^-$), a radical group (-R), and a hydrogen atom. The radical group (also called a side chain) is where each amino acid differs from others and that determines the amino acid properties. Each protein contorts into its own native state, a three dimensional structure determined by the amino acids in its chain. Just as the letters of our alphabet can be arranged in many ways to make words of different lengths, so too amino acids can be linked together differently to form many thousands of different proteins. Each protein has its own unique chain of amino acids, and each protein has its own purpose in a living thing. Proteins require a minimum of about 40 amino acids, but sizes range up to several thousand in multi-functional or structural proteins. The average length is about 300 amino acids. Also, very large aggregates can be formed from protein subunits.

The biological function of the protein is very dependent on its shape. A protein function is better understood when its shape is known. *Structural biology* uses techniques like X-ray crystallography or NMR spectroscopy to determine the structure of proteins. Solved structures can be deposited in the Protein Data Bank (PDB), a free resource containing thousands of proteins. Now there is an increasing interest in proteomics technologies because deoxyribonucleic acid (DNA) sequence information provides only a static snapshot of the proteins in which the cell might use its proteins, whereas the life of the cell is a dynamic process. Proteomics is the large-scale study of proteins, particularly their structure and functions. Food proteomics is one of the most relevant and fast developing areas in food science. This is a very dynamic field, developing and changing very rapidly in recent years.

Principles of proteomics, especially focused to understand

a) the proteome
b) purpose of proteomic evaluations, the extraction and fractionation techniques for proteins and peptides, followed by separation techniques such as 2-D electrophoresis and chromatography and the mass spectrometry applications
c) search in protein databases (Martínez-Maqueda *et al*. 2013).

Purpose of Food Proteomics

In broader term, Proteomics is defined as the total protein content of a cell or that of an organism. Proteomics helps in understanding of alteration in protein expression during different stages of life cycle or under stress condition. Likewise, Proteomics helps in understanding the structure and function of different proteins as well as protein-protein interactions of an organism. A minor defect in protein structure, its function or alternation in expression pattern can be easily detected using proteomics studies. This is important with regards to drug development and understanding various biological processes, as proteins are the most favourable targets for various drugs.

The application of proteomic techniques offers a way to investigate differences in the protein composition of different tissues within a specific animal or vegetable food type, as well as between different varieties of it. In addition it has the power to follow changes in the protein component of various tissues during growth, maturation and post-mortem or post-harvest, as well as downstream treatments such as cooking. According to the purpose, there are three kinds of proteomics.

a) Functional – Identification of protein-protein, protein-DNA, protein-RNA interactions affecting function
b) Structural – Identification of all interactions by metal ions, toxin, drugs etc. affecting protein structure
c) Differential – Determination of differences in protein expression (Dubey, 2013)

1. Steps in Proteomics (Frantisek, 2003)

The following steps are involved in analysis of proteome of an organism

i) Sample preparation
ii) Protein extraction
iii) Protein identification
iv) Protein characterisation

v) Bio-informatics

(i) Sample Preparation

Sample preparation has a profound effect on the final outcome of protein and peptide separation and their subsequent analysis.

Plant Tissues

Plant tissues contain relatively low amounts of proteins whose extraction is often difficult due to the presence of interfering compounds such as rigid cellulosic cell wall, storage polysaccharides, lipids and other contaminants that can cause protein degradation or modification. Plant tissues contain a wide range of protein, which vary greatly in their properties, and requires specific conditions for their extraction and purification.

Plant tissue are collected freshly from the plant, placed on a glass slide, checked under a dissecting microscope and any debris removed with the needle. The plant tissues are then cooled in an Eppendorf tubes and stored at -80^{o} C for later analysis (Sheoran, Ross, Olson and Sawhney, 2009) (or) frozen tissues were ground to fine powder in liquid nitrogen using a pre cooled mortar and pestle. The powdered tissue is stored in micro centrifuge tubes at – 80 °C until further use (Wang et al. 2003).

Animal Tissues

The extraction of proteins from animal tissues is relatively straightforward, as animal cells are enclosed only by a surface plasma membrane (also referred to as the limiting membrane or cell envelope) that is only weakly held by the cytoskeleton. They are relatively fragile compared to the rigid cell walls of many bacteria and all plants and are thus susceptible to shear forces. Animal tissues can be crudely divided into soft muscle (e.g., liver and kidney) or hard muscle (e.g., skeletal and cardiac). Reasonably gentle mechanical forces such as those produced by liquid shear may disrupt the soft tissues, whereas the hard tissues require strong mechanical shear forces provided by blenders and mincers. The homogenate produced by these disruptive methods is then centrifuged in order to remove the remaining cell debris.

Animals have many highly specialised tissues (e.g. liver, muscle, brain) that are rich in specific enzymes, thus facilitate their purification. This is not usually the case with plant enzymes, which may be present at low levels in highly complex protein mixtures.

Frozen tissues were ground to fine powder in liquid nitrogen using a pre cooled mortar and pestle. The powdered tissue was stored in micro centrifuge tubes at –80°C until further use (Skehel, 2003).

Fresh Seeds

The cotyledonary stage seeds are separated from the cones, collected in a vial then frozen and stored at -70^{o}C prior to extraction of protein (Zhen and Shi, 2011).

Cereals, Pulses, Nuts and Oil seeds

The seeds are crushed; the lipids are extracted with hexane in Soxhlet apparatus, hexane removed from the defatted meal at 40-50ºC in an oven with forced air circulation. Finally, the meal is finely ground to pass a 0.2 mm screen (Shela, Ines and Paulo, 1991).

Protein Extraction

A wide variety of extraction and fractionation tools for proteins and peptides are available based on their physicochemical and structural characteristics such as solubility, hydrophobicity, molecular weight and isoelectric point (Fig.2). Generally, different technologies focused on cell disruption, solubilization/precipitation and enrichment systems are needed to obtain the protein fraction of interest. Removal of interfering compounds (mainly lipids, nucleic acids, phenolic compounds, carbohydrates, proteolytic and oxidative enzymes, and pigments) is crucial. These procedures need to be optimized to minimize protein modifications and proteolysis, as well as to be compatible with subsequent analysis.

a. Cell Disruption Methods

The preparation of any biological material as a sample for proteomic analysis requires homogenization. Plants are generally more problematic for protein extraction because tissues are rich in proteases and other interfering compounds (Wang, Tai and Chen, 2008). Proteins are usually contained in protein bodies inside cell walls so cell disruption is required before they can be totally solubilized and extracted. The general procedure for sample preparation in this case strongly depends on the plant type, its fragment (leaf, fruit, seed, etc.), or even the stage of plant development.

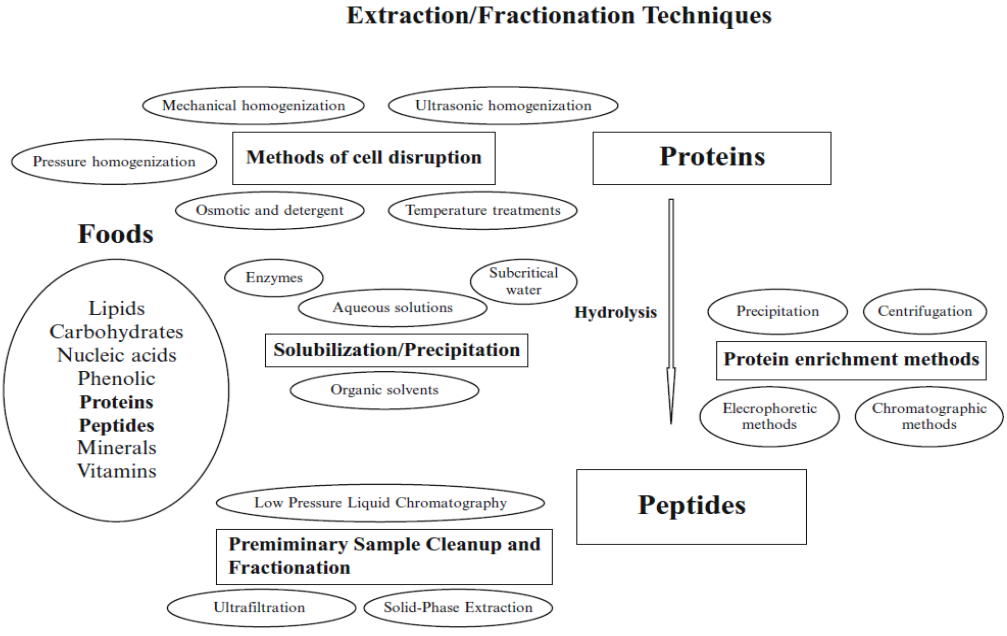


Fig. 2: Integrated extraction and fractionation techniques for proteins and peptides employed on proteomics in foods (Martínez-Maqueda et al. 2013).

Generally, disruption of the cell wall and protein release is crucial for analytical success. Various chemical and physical techniques can be used to destroy the cell wall. These techniques can be grouped into five major categories: mechanical homogenization, ultrasound homogenization, pressure homogenization, temperature treatments, and osmotic and chemical lysis (Table 1).

Table 1: Food/Plant cell disruption methods

Type of disruption	Procedure	Food	Tissue	Reference
Mechanical homogenization	Colloid milling and homogenization	Rice	Bran	Anderson and Guraya, 2001
	Centrifugal grinding and air dehulling	Pea, chickpea, lentil	Seed	Boye et al. 2010
	Cool mortar with lysis buffer	Rice	Embryo	Fukuda et al. 2003
	Cool mortar with Tris–HCl	Olive tree	Seed	Alche et al. 2006
	Wet-milling with sulphur dioxide	Sorghum	Seed	De Mesa-Stonestreet et al. 2010
Ultrasonic homogenization	Acoustic transducer	Soybean, rice	Root, sheath/hypocotyls, leaf	Toorchi et al. 2008
	Ultrasonic generator	Rice	Bran	Chittapalo and Noomhorm, 2009
Pressure homogenization	High pressure homogenization	Peanut	Seed	Dong et al. 2011
		Rapeseed	Seed	Barbin et al. 2011
		Tomato	Pollen	Sheoran et al. 2009
		Olive	Leaf	Wang et al. 2003
	Mortar and pestle with liquid N_2	Apple, banana	Mesocarp/Exocarp	Song et al. 2006
		Peanut	Seed	Liang et al. 2006
		Maize	Endosperm	Mechin et al. 2007
		Potato	Tuber	Delaplace et al. 2006
Temperature treatments	Pulverization in dry ice and grinding in liquid N_2	Grape	Berry cluster	Vincent et al. 2006
	Microwave, dry heating and parboiling	Rice	Bran	Khan et al. 2011
	Wet-milling with temperature	Sorghum	Seed	De Mesa-Stonestreet et al. 2010
Osmotic and chemical lysis	Hexadecyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS) or isopropyl alcohol (IPA)	*Lactococcus lactis* Strains	–	Doolan and Wilkinson, 2009

b. Protein Solubilization/Precipitation

The solubilization/precipitation process strongly affects the quality of the final results and thus determines the success of the entire experiment. Taking into account the immense variety of proteins and the huge number of interfering contaminants present in food-derived extracts, simultaneous solubilization of all proteins remains a great challenge. Each food sample requires a specific protocol that needs to be optimized to minimize proteolysis and modification of proteins (Bodzon-Kulakowska et al. 2007). For animal tissues, which have higher protein yields, various protein solubilisation buffers, including the use of chaotrophic agents, detergents, reducing agents, buffers and ampholites are used (Pedreschi et al. 2010). These are

- *Organic solvents (Table 2)*
- *Aqueous solutions (Table 3)*
- *Aqueous enzymatic extractions (Table 4)*
- *Subcritical water (Table 5)*

b. Protein Separation and Enrichment

Once the protein fraction has been separated from other interfering substances or compounds, increasing the concentration of the proteins is of the great interest. The separated proteins are concentrated by centrifugation, precipitation, electrophoresis and chromatography methods.

Centrifugation

It is the first step to separate different cell substances where proteins of interest are locally concentrated, for instance, mitochondria, membrane, or nucleus. Cellular homogenate is separated into different layers based on the molecular weight, size and shape of each component. The proteins of interest are solubilised, enriched and fractionated prior to MS analysis. The separation takes place based on the coefficient of sedimentation of the proteins expressed in Svedberg units.

Precipitation

The protein precipitation is done by

- Salting out method

  In salting out method, the concentration of different salt precipitants, ammonium sulphate or sodium chloride varies from one protein to another (Deak et al. 2006).

- Immuno-precipitation

  In immune-precipitation, antigen binds to its specific antibody to form antigen-antibody complexes. It offers high recovery of protein and used for food allergens identification (Pastorello and Trambaioli, 2001).

Electrophoresis

Electrophoresis separates mixtures of proteins based on charge, charge/mass ratio, size or shape. This technique is mainly used as an analytical and preparative tool, especially one-dimensional separation, often employed as a pre-fractionating technique (Guttman et al. 2004; Jorgenson and Evans, 2004). Often, laboratories dedicate one-dimensional gel

electrophoresis (1DE) to evaluate the outcome of protein purification preceding the analysis by 2DE (Chen et al. 2007). The 2D gel electrophoresis is applied for separation of proteins on the basis of their isoelectric points in one dimension [A special detergent is added to the solution of proteins to release the tangle of protein chains. These are then separated on the basis of their natural charge (their *isoelectric point*) by putting them in a narrow tube of polyacrylamide gel that contains a pH gradient. An electric current is applied down the length of the strip of gel. Over time, the different proteins move with the current, but stop at different places in the gel] and molecular weight on the other [Proteins are separated on the

Table 2: Organic solvents

Solvent (s)	Food	Tissue	Reference
Acetic acid/urea/Cetyl trimethyl ammonium bromide	Rice	Bran	Hamada, 1997
Aqueous ethanol	Distiller's grain	Grain	Cookman and Glatz, 2009
Aqueous isopropanol	Soybean	Seed	Natarajan et al. 2009
	Rapeseed	Seed	Barbin et al. 2011
Ethanol	*Saccharina japonica*	-	Kim et al. 2011
Glacial acetic acid	Sorghum	-	De Mesa-Stonestreet et al. 2010
Phenol	Tomato	Pollen grain	Sheoran et al. 2009
	Potato	Tuber	Delaplace et al. 2006
	Aloe vera	Leaf	He and Wang 2008
	Soybean	Seed	Natarajan et al. 2005
	Barley	Root	Hurkman and Tanaka, 1986
Phenol/ammonium acetate	Avocado/tomato/ orange/banana	Fruit	Saravanan and Rose, 2004
	Banana	Leaf	Carpentier et al. 2007
	Grape	Fruit	Vincent et al. 2006
	Pear	Fruit	Pedreschi et al. 2007
	Apple/strawberry	Fruit	Zheng et al. 2007
Phenol/methanol-ammonium acetate	Coniferous	Seed	Zhen and Shi, 2011
	Banana/apple/potato	Tissues	Carpentier et al. 2005
Sodium dodecyl sulphate/ acetone	Coniferous	Seed	Zhen and Shi, 2011
	Potato	Tuber	Delaplace et al. 2006
Sodium dodecyl sulphate/ TCA/acetone	Apple/banana	Tissue	Song et al. 2006
TCA	Bean	Anther	Wu and Wang 1984
TCA/acetone	Citrus	Leaf	Maserti et al. 2007
	Soybean	Seed	Natarajan et al. 2006
	Soybean	Leaf	Xu et al. 2006
	Coniferous	Seed	Zhen and Shi, 2011
	Tomato	Pollen grain	Sheoran et al. 2009
	Aloe vera	Leaf	He and Wang, 2008
	Apple/banana	Tissues	Song et al. 2006
	Aphid	Tissues	Cilia et al. 2009
TCA/acetone/phenol	Olive	Leaf	Wang et al. 2003
	Bamboo/grape/lemon	Leaf	Wang et al. 2006
	Apple/orange/tomato	Fruit	Wang et al. 2006
Thiourea/urea	Soybean	Seed	Natarajan et al. 2005
	Apple/banana	Tissues	Song et al. 2006
Tris–HCl buffer	Tomato	Pollen grain	Sheoran et al. 2009

Table 3: Aqueous Solutions

Solvent (s)	Food	Tissue	Reference
Acid (TCA or carboxy methyl cellulose)	Vegetables	Tissues	Massoura et al. 1998
Alkali sodium and calcium salts	Vegetables	Tissues and seeds	Ghaly and Alkoaik, 2010
	Meat and fish	Tissues	Liang and Hultin, 2003
	Rice	Bran	Jiamyangyuen et al. 2005

Table 4: Aqueous Enzymatic Extraction

Enzyme	Food	Tissue/sample	Reference
Alcalase TM	Rice	Bran	Hamada, 2000
Alcalase 2.4 L	Rapeseed	Seed	Zhang et al. 2007
	Peanut	Seed	Wang et al. 2008a
	Peanut	Roasted seed	Zhang et al. 2011
Alcalase + Protamex (1:3)	Tea Leave	Pulp	Shen et al. 2008
Alkaline protease	Rice	Broken rice	Hou et al. 2010
Flavourzyme	Rice	Bran	Hamada, 2000
Glucoamylase	Lentil	Bean	Bildstein et al. 2008
Neutrase 1.5MG	Coconut	Meat	Sant'Anna et al. 2003
Olivex + Celluclast	*Guevina avellana*	Pressed cakes	Moure et al. 2002
Papain	Rice	Broken rice	Hou et al. 2010
Pectinase + Protease P	Rice	Bran	Tang et al. 2003
Phytase	Rice	Bran	Wang et al. 1999
Protex 6 L	Distiller's grain	Grain	Cookman and Glatz, 2009
	Lupin	Seed	Jung, 2009
	Soybean	Seed	Jung, 2009
	Soybean	Flakes	De Moura et al. 2011
Protex 7 L	Sesame	Seed	Latif and Anwar, 2011
	Moringa oleifera	Seed	Latif and Anwar, 2009
Viscozyme L	Coconut	Meat	Sant'Anna et al. 2003
	Oat	Bran	Guan and Yao, 2008
Viscozyme L + Cellulast 1.5 L	Rice	Bran	Tang et al. 2002
		Bran	Ansharullah et al. 1997
Xylanase	Rice	Bran	Wang et al. 1999
Xylanase + Phytase amylase	Rice	Bran	Wang et al. 1999

Table 5: Subcritical Water

Solvent	Food	Tissue	Reference
Water	Defatted flaxseed	Meal	Ho et al. 2007
	Rice	Bran	Watchararuji et al. 2008
	Soybean	Meal	Fabian and Ju, 2011

basis of size, by using a technique called *sodium dodecyl sulfate polyacrylamide-gel electrophoresis* (SDS-PAGE)]. The narrow gel containing the separated proteins is again subjected to an electric current, but in a direction that is at a right angle to the direction that used in the first step. Each protein then migrates away from its fellows of different size to form a discrete spot. The gel is stained with a dye to make the spots of protein visible. Spots are detected using fluorescent dyes or radioactive probes.

Chromatography

Liquid chromatography (LC) techniques are most commonly used in proteome pre-fractionation prior to in-depth analysis. The separation of different proteins is achieved according to their charge (Ion – Exchange Chromatography - IEC), hydrophobicity (Reverse phase Liquid Chromatography - RP-LC), size (size-exclusion chromatography - SEC), or specificity (Affinity Chromatography - AC). In some cases, chromatographic methods can also be used to eliminate some interference substances (e.g., salts) coming from previous enrichment steps.

(ii) Protein Identification

The separated protein spots on gel are excised and digested in gel by a protease (eg. trypsin). The eluted peptides are identified using mass spectrometry. Steps involved in protein identification are

a. Protein digestion
b. Peptide extraction and fractionation

a. Protein Digestion

Once the proteins have been isolated from interfering compounds (other food components such as lipids, nucleic acids, phenolic compounds or carbohydrates), they are usually analyzed by 1D or 2D SDS-PAGE, depending on the complexity of the sample. Direct digestion of a mixture of proteins is adequate when a broad survey of the identifiable protein components is desired or to minimize the loss of peptides by binding to the poly acryl amide when characterizing post-translational modifications (Kinter and Sherman, 2005). Different proteolytic agents are used for protein digestion, including enzymes such as trypsin, different endoproteases (Lys-C, Arg-C, Asp-N, Glu-C) or chymotrypsin, as well as chemical reagents such as hydroxylamine or cyanogen bromide. The specificity of the amide bond or bonds cleaved by these reagents allows the obtaining of specific peptides that facilitate the interpretation of their mass spectra and database search. Trypsin is certainly the most popular reagent because it shows many advantages compared to other enzymes and chemical reagents, in addition to its relatively low cost of production and high purity. Two types of digestion are

- In – gel digestion
- In – solution digestion

- **In-gel digestion**

The in-gel digestion is part of the sample preparation for the mass spectrometric identification of proteins in course of proteomic analysis. The method was introduced in 1992 by Rosenfeld et al. Despite innumerable modifications and improvements, the basic elements of the procedure remain largely unchanged. The in-gel digestion primarily comprises the four steps destaining, reduction and alkylation (R & A) of the cysteines in the protein, proteolytic cleavage of the protein and extraction of generated peptides.

- **In-solution digestion**

Proteins in solutions are denatured, their disulfide bonds are reduced, and the Cys residues are alkylated. The protein samples are incubated with trypsin for several hours, and the resulting peptides can be analyzed by MS.

b. Peptide extraction and Fractionation

In general, food samples are first subjected to a preliminary sample cleanup step to remove interfering substances and then, different fractionation steps are applied, as has been widely revised (Gonzalez de Llano et al. 2004; Asensio-Ramos et al. 2009; Hernandez-Ledesma et al. 2012).

- **Extraction and preliminary sample cleanup**

Peptide extraction is usually followed by a preliminary sample cleanup for removal of other food components (i.e., proteins, lipids). Deproteinization, the most important preliminary cleanup procedure in peptide analysis, is carried out by precipitation of protein using several agents. Deproteinization could also act as a fractionation procedure for peptides because their solubility depends on the precipitating agent and its proportion (Cheng et al. 2010). After precipitation, centrifugation and filtration methods are used to separate proteins from soluble peptides. In addition, the application of heat treatments or ultracentrifugation steps at high speed to eliminate the proteins has been reported (Gomez-Ruiz et al. 2007; Ho et al. 2010).

- **Fractionation**

Peptides are fractionated based on their molecular size by ultra filtration, low pressure size exclusion chromatography, low pressure ion exchange chromatography solid phase extraction methods.

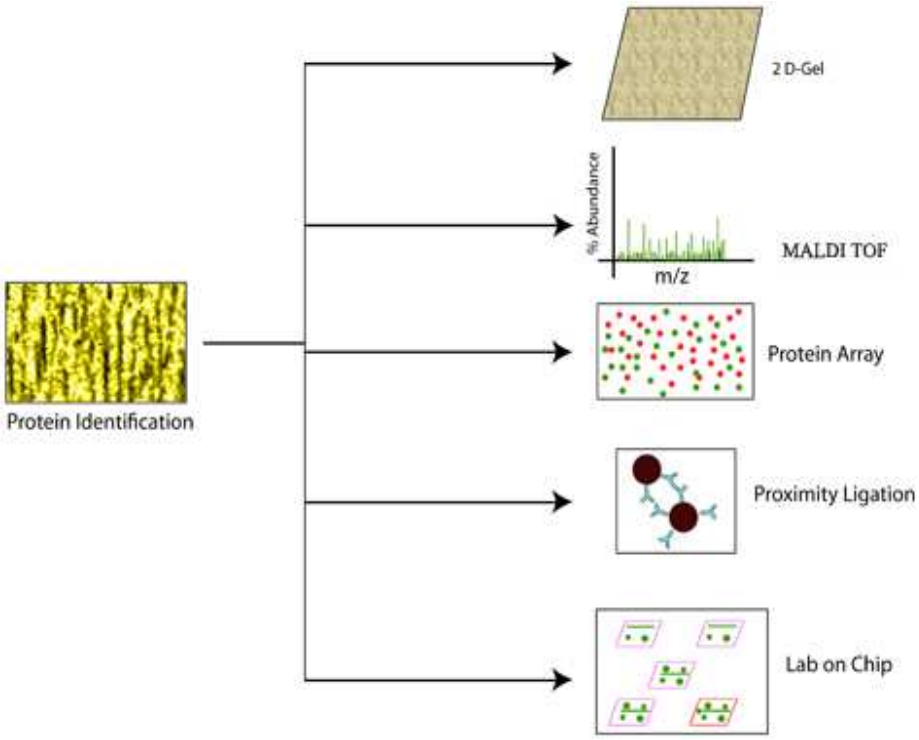


Fig 3. Techniques involved in protein identification

(iii) Protein Characterisation

a. Peptide Analysis by Mass Spectrometry

In practice, whether a large or small fraction of the peptides generated from any protein is detected depends on many variables: the amount of that protein present in the original sample; the efficiency of any protein extraction, digestion, and peptide extraction; the presence of other proteins and other impurities; and the sensitivity and performance characteristics of the mass spectrometer and its mode of ionization, mass separation, and ion detection.

Mass spectrometers employed in proteomic analysis use either matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) but they vary widely in their operation and performance characteristics. Early database searching was based on low-resolution linear MALDI-time-of-flight (MALDI-TOF) giving a mass accuracy of perhaps ±2 Da. However, this is no longer acceptable because good mass accuracy increases the reliability of database searching by limiting the possible compositions of peptides for any given mass. A delayed extraction MALDI-TOF instrument with reflectron should give better than 50 ppm mass accuracy, and significantly better (~10 ppm) may be achieved with careful internal calibration. ESI has been the standard ionization method for liquid chromatography (LC)-MS and LC-tandem MS (MS/MS), although separated fractions can be deposited onto a MALDI target for either on-line or off-line analysis. Fourier transform MS gives the ultimate performance with mass accuracy of perhaps one ppm, but such instruments are more expensive, more technically demanding, and until now their deployment has been mostly limited to large MS facilities.

b. ***Peptide analysis by database searching***

There are three approaches to protein identification based on peptide analysis by database searching

- Peptide mass mapping or mass fingerprinting
- Collusion – induced dissociation (CID) spectra
- De novo sequencing

- **Peptide Mass Mapping or Mass Fingerprinting**

This relies upon a comparison of the experimentally determined MS peak mass values with the predicted molecular mass value of the peptides generated by a theoretical digestion of each protein in a database.

- **Collusion – induced Dissociation (CID) Spectra**

The use of MS/MS and CID is becoming the accepted standard for protein identification and is steadily replacing peptide mass fingerprinting, although the quality of tandem data varies considerably with instrument type. A number of web sites offer free access to web-based database searching programs for peptide mass fingerprinting and the identification of sequence tags, all of which provide other tools as well, such as programs for calculating isotope patterns, predicting enzyme digestion patterns, and theoretical prediction of CID fragments. An enumeration of all the current search engines is a moving target, but well-known web-based examples include tools on the ExPASy proteomics server provided by the Swiss Institute of Bioinformatics (www.expasy.ch/tools), Mascot from Matrix Science (London, UK; www.matrixscience.com), and Protein Prospector provided by the University of California (San Francisco, CA; prospector.ucsf.edu). Systems supplied by instrument manufacturers include Sequest from Thermo-Finnigan (San Jose, CA) and Spectrum Mill from Agilent Technologies (Palo Alto, CA).

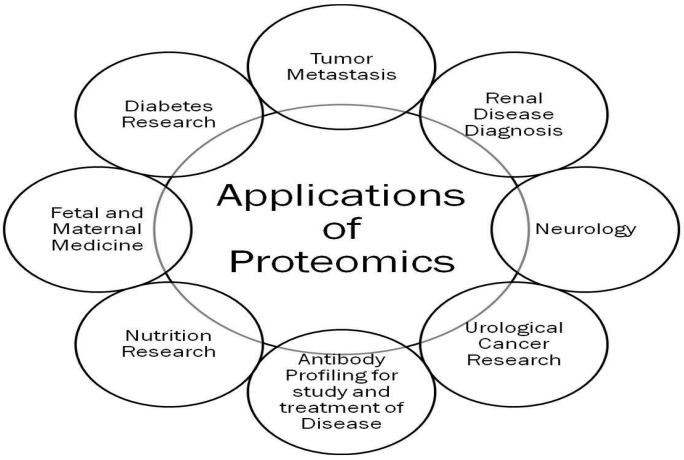
- ***De novo*** **Sequencing**

De novo (peptide) sequencing for mass spectrometry is typically performed without prior knowledge of the amino acid sequence. It is the process of assigning amino acids from peptide fragment masses of a protein. *De novo* sequencing has proven successful for confirming and expanding upon results from database searches.

(iv) Bio-informatics (cross reference of protein information with genomic databases)

Determined amino acid sequence is finally compared with available data base to validate the proteins. Several online tools are available for proteomics such as Mascot, Aldente, Popitam, Quick mod & Peptide cutter etc.

APPLICATION OF PROTEOMICS IN FOOD SCIENCE AND NUTRITION



1. Screening for novel functional bioactives - Availability of new rapid screening methods for detection of bioactivity.
2. Safety evaluation of food ingredients - Evaluation of absorption, body distribution and metabolism of food ingredients.
3. Detection and control of food spoilage or pathogenic microorganisms - Identification of biomarkers (genes, proteins, metabolites) representative for specific food spoilage and / or pathogenic microorganisms.
4. Efficacy testing of bioactive functional food ingredients - Changes in gene expression and proteome relevant to the states or treatment of certain diseases.
5. Food allergy - Identification of allergic protein through sophisticated proteomics based on recognition of specific posttranslational modification and digestion-resistant peptide features.
6. Quality and authenticity of foods - Proteome of certain food (wheat, wine and fish) can be used to authenticate food origin or food quality.
7. Production of food ingredients - The yield of bioprocess (production of amino acids, carbohydrates etc.) may be controlled through metabolome / proteome of microorganisms used for such productions.
8. Food processing - Proteomics and / or metabolomics of starter culture of fermentation processes (beer, cheese, sausage etc.) can be used to predict the quality of the fermented end- product.
9. Food proteomics applicability deals with quality issues related to post-mortem processes in animal foods and quality traits for a wide variety of foods such as meat, fish, dairy, eggs, wine, beer, cereals, fruits and vegetables, but also for the identification of bioactive peptides and proteins which are very important from a nutritional point of view. Furthermore, consumers are now extremely susceptible towards food safety issues and proteomics can help in assuring different safety aspects including food authenticity, detection of animal species in the food, markers of pathogenic microorganisms.
10. The proteomic evaluation of food proteins presents a unique set of challenges and opportunities. In addition to post-translational modifications produced *in vivo*, food proteins are subjected to a wide range of post-harvest/post-slaughter environmental and processing insults prior to consumption. These modifications include side-chain oxidation, cross-link formation and backbone cleavage, and critically influence key food properties such as shelf-life, nutritional value, digestibility and health effects. A

profound understanding of proteomics, protein modifications and redox chemistry has allowed us to pioneer the application of redox proteomics to foods.

11. Food proteomics deals with the full spectrum of protein-containing foods, including dairy, meat, seafood and cereal proteins (Clerens, Plowman and Dyer, 2012).

CONCLUSION

Proteomics has broad applications in all the aspects of life sciences including several practical applications as drug development against several diseases. Difference in expression protein expression profile of normal and diseased person may be analyzed for target protein. Protein to gene may be predicted. Once protein/gene is identified, function may be predicted. This can help in disease management/drug development.

Human food is a very complex biological mixture; food processing and safety are very important and essential disciplines. The use of proteomics in food technology is presented, especially for characterization and standardization of raw materials, process development, detection of batch-to-batch variations and quality control of the final product. Proteomics technology using different high-performance separation techniques such as two-dimensional gel electrophoresis, one-dimensional and multidimensional chromatography, combined with high-resolution mass spectrometry has the power to monitor the protein composition of foods and their changes during the production process.

The application of integrated nutrigenomics approach in nutritional sciences, allow for accelerated implementation of mechanistic knowledge in food design. The application and modification of proteomic approaches to analyse the complexity of food protein modification is anticipated to become increasingly important in the area of general food science, quality assurance and product differentiation. Further attention is paid to the aspects of food safety, especially regarding biological and microbial safety and the use of genetically modified foods.

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Food Security and Resilience among Mobile Pastoral and Settled Community around Lake Chad in Sahel: An Overview

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ABSTRACT

Food Insecurity in Africa and particularly in Sahel area is the most brake of development. It increasing levels of malnutrition and jeopardizing efforts to improve public health. This study aimed to investigate food accessibility, resources availability and strategies developed by communities against periods of supply shortfall. A cross-sectional household survey was carried out among 300 women and 150 men in mobile pastoralist and sedentary communities. It is completed by data collected in markets and veterinary care facilities. The mobile pastoralist which is the target community develops strategies against food insecurity by diversifying their income sources, storages food and creates information networks. Women play an important role by sale of dairy products and petty trade. The main source of income for mobile pastoralists remained the livestock management. The income did not only contribute to improve their livelihood, nutritional status or health and wellbeing of their family. It was largely taken off for payments to local authorities, land owners, and intermediate tradesmen. The prevailing institutional setting was inadequate and unable to protect mobile pastoralists and rural sedentary communities, who remain the most vulnerable groups.

KEYWORDS: Mobile Pastoralist Food Security & Sahel Area

INTRODUCTION

In Sahelian countries of Africa, livestock contributes to 44% of agricultural gross domestic product (GDP) and 34% of household income (Zoundi and Hitimana, 2008). Despite this important economic value, food production is generally weak and cannot cover the needs of local populations. It represents 25 kg of milk equivalent per person per year and 14 kg of meat per person per year, which is only a fraction compared to developed countries such as Switzerland with 232 kg of milk equivalent per person per year and 59 kg of meat per person per year (FAO, 2005). The low level of production leads to large imports of animal proteins. Up to 90% of West Africa's urban milk demand is covered by imported powder milk (Pinaud, 2007; Pinaud and Corniaux, 2009), associated with important losses in foreign currency with annual costs of 93 million USD in Senegal and 40 million USD in Mali (1 USD = 454 FCFA, Chadian currency, Communauté Financière Africaine Franc) (Dieye et al., 2005;

Collectif Alimentaire, 2006). Cereal production in the Sahel is also below actual needs. The total crop production in 2007/2008 noted a deficit of 913'100 tonnes which were compensated by importations into Sahelian countries (CILSS, 2008). The causes are especially structural and circumstantial, but more important are the repeated droughts, which exacerbate the poor nutritional status, particularly among rural and nomadic communities. For example, the famine of 2005 affected 2.5 of the 11.5 million people in Niger (Centers for Disease Control and Prevention, 2006).

In Chad, livestock represents 53% of agricultural GDP and 30% of export value (another 50% of export value comes from petrol) (Ministère de l'Elevage et des Ressources animales Tchad, 2008). Similar to the entire Sahelian region, animal production for local consumption remains weak. It varies around 24 kg of milk equivalents per person per year and 13 kg of meat per person per year (FAO, 2005) with significant seasonal variation. Daily milk production in greater N'Djamena was estimated at 50'000 litres in May, at the end of the dry season, and at 300'000 litres per day in August, during the rainy season. (Koussou, 2008). The high volume of milk in the rainy season can generally not all be used because of the lack of infrastructure for conservation. This results in Chad importing up to 40% of its dairy product demand (Koussou, 2008). The cereal deficits are frequent and production is also highly variable. Chad registered a cereal deficit of 216'900 tonnes in 2005 and a gain of 75'900 tonnes of cereal in 2007 (CILSS, 2005, 2008).

These unfortunate realities cause Sahelian countries to implement different institutional arrangements for preventing and managing droughts and natural crises. The permanent inter-state committee for drought control in the Sahel was created in 1973 (CILSS; Comité permanent inter-états de lutte contre la sécheresse au Sahel). Its mandate was *"to invest in the research for food security and to fight against the effects of drought and desertification for a new ecological balance"*. At the national level, Chad has a national program of food security (PNSA: Programme national de sécurité alimentaire). Its objective is to fight against famine and food insecurity (Ministère de l'Agriculture et al., 2005). Since 1989, a stock of food security for emergency has existed (order N°49/MSAPS/DG/89). In 1995, an action committee was formed to address food security and the management of disasters (CASAGC) (order N°30/MAE/CAB/95).

The World Food Summit of 1996 defined food security as existing "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life".

In 1998, an information system for food security and early warning (SISAAR) was implemented. Its mission is to centralise information on food security and to assure the permanent follow-up of food and nutritional patterns in Chad. Development projects focusing on rural and pastoral issues have been initiated beginning in the 1990s. However, the various institutional frameworks put in place have a general scope and do not emphasise specific solutions for the remote and mobile communities.

This present chapter include food accessibility, resources availability, strategies developed against periods of supply shortfall and institutional constraints in Chad (Weibel et al. 2011). It is a companion chapter to parallel studies on demographics and malnutrition among children under five year old and women in the same study population (Bechir et al. 2010).

Survey protocol

The survey was conducted in the region of Hadjer-Lamis on the southern border of Lake Chad among mobile pastoralist and settled rural communities. It involved 45 randomly selected camps from 3 mobile pastoral communities (Foulbe, Dazagara and Arab) and 3 villages of settled communities (Gredaya, Sidje and Baltram) which were main trading places between mobile pastoralists and settled communities in this area. These villages were composed of many settled communities of which the most important were Kanembou, Arabs, and inhabitants who immigrated during the 1980s from central Chad (e.g. Hadjaraï and Bilala) and southern Chad (e.g. Sara, Massa).

Data were collected at three levels. Firstly, at the household level semi-structured interviews were held among 300 mobile pastoralist women and 150 mobile pastoralist men. The same numbers were interviewed among settled communities. The sample size was indirectly calculated based on children less than five years old involved in a parallel study of malnutrition. In this study, the number of children was calculated considering a weighted prevalence of malnutrition of 28%, an intra-class correlation of 0.2 (clustering) and a confidence level of 95% and a standard error of 5% (Bechir et al. 2010). The interviewed women were the mothers of these children, mentioned above. The number of men heads of household was lower because of their frequent absence from the household during the interview period or due to polygamy that contributed to reduce the men to women ratio. The nomadic camps were selected randomly along transects using a geographical positioning system (GPS) (Weibel et al., 2008).

At a second level, prices of major goods on the weekly market were recorded, and interviews were conducted with key informants (market managers, sales representatives). At a third level, data from health records for humans and animals were collected from health facilities in the study area. The collected data was double entered into Access software (Microsoft 2003) and validated with Epi Info 2000 (version 3.5.1 2008). STATA software (Statistic data analysis version 10.1) was used for descriptive data analysis.

Finding

A total of 682 women were involved in this study among which 348 were mobile pastoralists and 334 lived in sedentary communities. Three hundred and forty-nine heads of household (men) were interviewed, among which 167 were mobile pastoralists and 182 lived in sedentary communities. (Table 1)

Table 1: Number of interviewed persons

Sample	Pastoralist communities	Sedentary communities	Total
Women	348	334	682
Men	167	182	349

The average age of the women was 28.5 years (95% CI: 24.9-26.4) for the mobile pastoralists and 25.6 years (95%CI: 24.9-26.4) in the sedentary communities.

The youngest women were 14 years old among nomadic and sedentary communities. Among nomadic and sedentary men the average ages were, respectively, 42.4 (95% CI: 40.5-

44.3) and 44.6 (95% CI: 43.2-46.1) years. The ethnic distribution among mobile pastoralist women was: 58% Foulbé, 20% Dazagara and 22% Arabic. Among sedentary communities, there were proportions of settled people coming from mobile pastoralist ethnic groups, namely 7% Foulbé, 6% Dazagara and 23% Arabic. The other communities were Haoussa 16%; Kanembou 14%, southern Chad (Sara, Massa) 12% and central Chad (Hadjarai, Boulala and Kouka) 21%.

More than 95% of mobile pastoralist and settled women were married. Single, divorced or widowed women represented less than 2% each. Slightly over one third of all married women lived in polygamous relationships, of which 40-45% were first, 47-49% second and 5-10% were third women. Mobile pastoralist women had 4.8 (95% CI: 3.9-4.5) children on average and 3.4 (95% CI: 3.2-3.6) among settled communities. Among mobile pastoralists, only one out of 348 women attended primary school, whereas one third of sedentary women were educated (Quranic school (51%), primary school (28%), secondary school (20%), professionals (1%).

Women's income activities

Most mobile pastoralist and sedentary women had household and income generating activities. However, there was more diversification of activities among sedentary women compared to mobile pastoralists. Mobile pastoralist women sold curdled-milk and butter, whereas sedentary women were engaged in petty trade (46%), handicrafts (16%) and other activities, e.g. in local restaurants (35%). Only 3% of sedentary women sold milk or milk products. Income from these activities was mainly used for household needs.

Main activities of men

In addition to their own livestock husbandry, mobile pastoralist men were engaged in animal trading (21%), in animal keeping (15%) or as traditional healers (marabout) (8%), but half of them also participated periodically in agricultural work. Main activities of sedentary men were farming (69%) and trading (10%). Less than 2% dealt with animal husbandry, but 20% had secondary activities as public servants, soldiers, tailors or butchers.

Animal and dairy resources

It is difficult to evaluate numbers of animals among mobile pastoralists. Data was collected from interviews and vaccination registers. Reported animal numbers from semi-structural interviews were on average 25 (95% CI: 23-27; n=158) zebu cattle per household. Average numbers from vaccination register were 55 (95% CI: 50-61; n=170). Three out of four households raise zebu cattle and small ruminants (sheep and goat) with 23 (95% CI: 21-25) small ruminants per household. In 56% of the households women milked more than 7 cows, yielding 1-2 litres of milk per day in the dry season. Among sedentary households, 6% had an average of 5 zebus (95% CI: 2-9), and 19% had on average 9 small ruminants (95% CI: 13-63). (Figure 1)

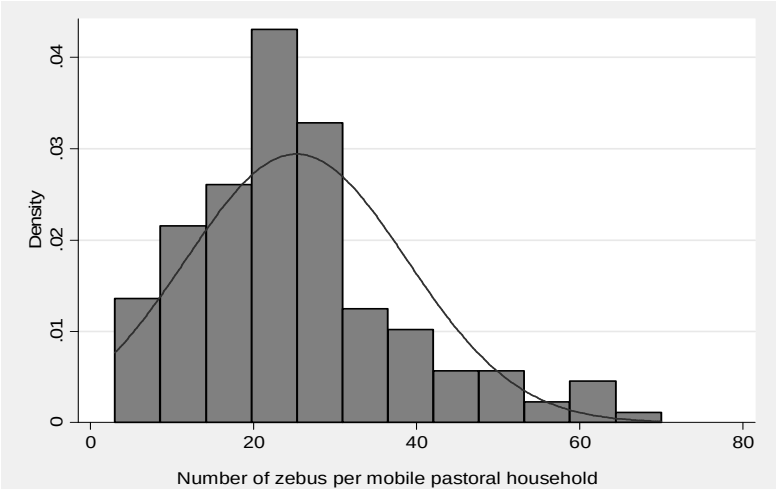

Figure 1 Distribution of zebus in nomadic households

Crop resources

The cereal (corn) production among sedentary households was on average 2.8 (95% CI: 2.5-3.2) tonnes per household per year. Up to 11% of mobile pastoralists were also involved in agricultural activities in the Lake Chad area producing on average 1.1 (95% CI: 0.8-1.5) tons of cereal on average. (Figure 2)

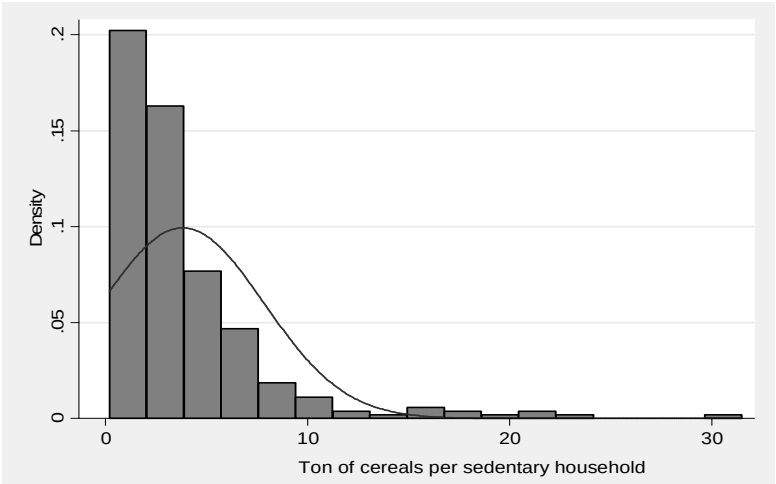

Figure 2 Distribution cereals in sedentary households

Main food price variations in the market of Gredya

Corn, fermented milk, and melted butter were the main local commodities. The prices of those items were quite variable according to the demand of weekly markets and seasonal variation. Corn was the main cereal in the Lake Chad area. The average yearly price observed in 48 weekly markets was 19'313 FCFA (95% IC: 17'777-20'848) for 100kg of corn or 0.43 USD per kg (1 USD = 447 FCFA[1]). World market prices vary around 8 USD per bushel

[1] Accessed on April 9, 2011 www.oanda.com,

(1 bushel = 25.4 kg) or 0.31 USD per kilo[2], which is less than the market price in the study area. The lowest price was observed in December, 10'000 FCFA, and the high in August, 29'000 FCFA. The average yearly price of fermented milk was 120.8 FCFA (95% IC: 108-134) for one litre, with a minimum of 25 in August and a maximum of 225 in February. The average yearly price of melted butter was 2'597 FCFA (95% IC: 2'492-2'701) for one litre with a minimum of 1'950 in August and a maximum of 3'330 in May. Figure 3 shows the variation of monthly prices of corn, fermented milk and melted butter. (Figure 3:).

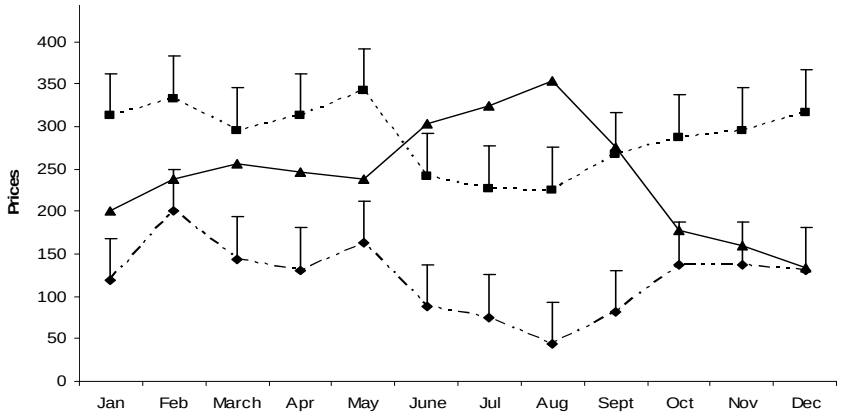

Figure 3: Yearly food price variation in the market of Gredaya: Diamonds: 1 litre of fermented milk, Squares: 100g of Butter, Triangle: 1 kg of Corn. Prices in FCFA.

Livestock trade

The main sources of livelihoods of mobile pastoralist communities are from livestock and the sale of dairy products. A total of 24 weekly markets were followed in Karal between February and July 2007. During this period, 737 animals were sold, among them 581 zebus (79%) and 156 small ruminants (21%). Up to 80% of zebus sold were transported to Nigeria and the remainder were reintroduced into the local herds, with 1% being transported to the capital N'Djamena and 0.5% to Cameroon. More than half of the small ruminants (54%) were taken to N'Djamena, with 44% reintroduced into local herds and 2.5% sold to local slaughterhouses. The average sale's price in this period was 221'875 FCFA (95% CI: 214'598-229'153) for cattle and 23'230 FCFA (95% IC: 22'218- 24'242) for small ruminants. Figure 4 shows the seasonal trend of livestock prices. The main reason given for animal sales was to buy food stock. Other reasons for sales were socio-cultural events (marriage or death), conflict resolution and management of the pastoral area. (Figure 4)

[2] Accessed on April 9, 2011 http://news.tradingcharts.com/futures/9/2/156640329.html,

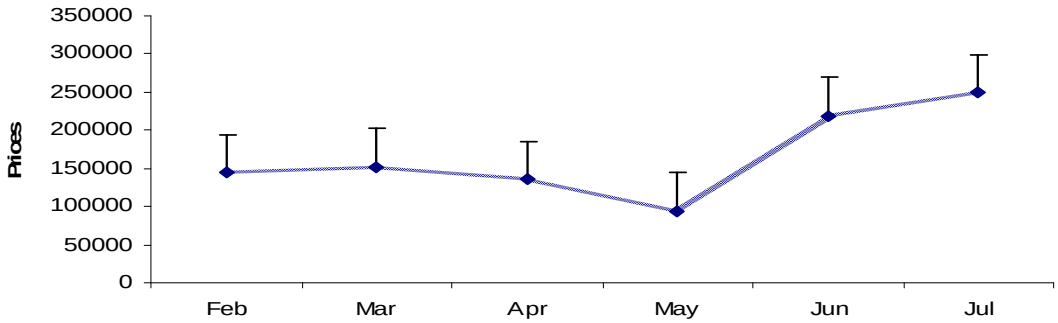

Figure 4. Average monthly Zebu cattle price (FCFA) variation in the weekly markets of Karal. Vertical bar is upper 95% confidence limit.

The sale of raw milk is not common in this region because of the conservation problem for Arabic and Dazagara communities and the traditional prohibition of raw milk consumption among Foulbe communities. The milk sold (rouaba) is skimmed and fermented. It can be conserved as long as two weeks. It is sold by mobile pastoralists and collected by intermediary traders in cans of 40-60 litres prior to being routed to N'Djamena. The weekly market of Baltram is the most important one and routes 15'000 to 20'000 litres per week. Only women participated in this activity. Outside of the weekly market, fermented milk was exchanged against cereals at the volume in *bogodos* (a cup with a volume of about one litre). (Figure 5 & 6)

Figures 5 & 6: Acid milk collected in the market of Baltram and routed to N'Djamena

Food stock

Mobile pastoralists, although mobile, always tended to constitute their food stocks. Almost all mobile pastoralists were stocking food, against only 59% among sedentary communities. The stocked foods were exclusively cereals conserved in bags of 100kg and stored by friends in neighbouring villages (16%) or on hire (84%). The hiring cost was 500 FCFA (1.2 USD) per bag without a limit of duration. Only 1.5% of nomadic had their stock in the camp. The average number of stocked bags (100kg) was 9.9 (95% CI: 8.8- 11.1) among nomadic and 26 (95% CI: 23.3-28.6) among sedentary. The best period for stocking was the harvest period, corresponding to the month of November. In this period, animals were expensive and cereals were cheaper. Sixty two (62) percent of pastoralists waited until this time to sell animals and buy cereals.

Current food expenses outside of the stocks

The monthly food expense without stocking cereals and auto-consumption was on average 17'300 FCFA per household (35 USD) among mobile pastoralists and nomadic communities and 43'741 FCFA among sedentary communities (89 USD).

Information and market accessibility

Transhumance and weekly markets were regulated by information exchange. Those exchanges happened in the market, during social events and also during meetings of traditional chiefs.

Radio broadcasting was also a source of reception and diffusion of information. Seventy eight percent of households had a radio receiver, compared to 94% of sedentary communities. Information was easily accessible because of the general use of the local Chadian Arabic language in the Lake Chad area. Mobile phones arrived recently in this area and represent a real revolution, facilitating transhumance by enabling mobile pastoralist scouts to inform others on the availability of good grass and water over large distances. Devices for charging batteries were developed locally. The greatest distance between mobile pastoralist camps and the regional big market of Massakory was about 150 km. smaller weekly markets in this area took place in the three investigated villages plus others. (Figure 7)

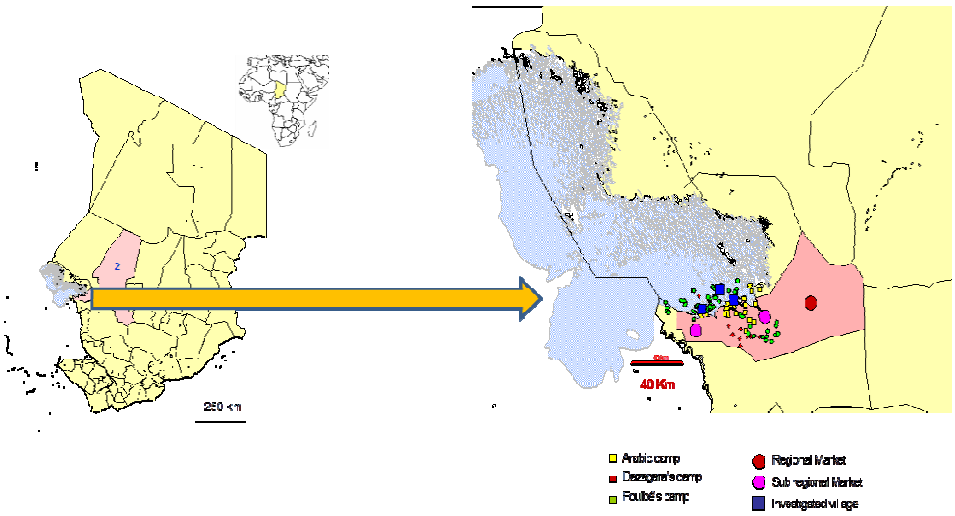

Figure 7: Map of investigated camps and weekly markets in the villages

Factors affecting food availability and accessibility

Despite the relatively favourable market access and availability of natural resources, malnutrition in the same study population is rampant (Bechir et al. 2010). Many of the factors factors listed below contribute, often in an interdependent way to the current vulnerability and malnutrition of mobile pastoralist and settled communities in Lake Chad.

Animal disease constraints

Lake Chad is infested by parasites and insects which reduce optimal livestock production. This is an important factor pushing pastoralists out of the Lake Chad area in the wet season. The most important insects are tabanids, which disrupt animals from browsing during the day and act also as vectors of hemoparasites. *Fasciola hepatica* is a highly endemic parasite affecting cattle production.

Human and animal overpopulation around Lake Chad

The Lake Chad region is one of the most densely populated areas of the Sahel. We found all nationalities of the neighbouring countries and beyond. Livestock breeders came from many parts of Chad, Kanem, Chari-Baguirmi and Bahr-El-Gazal. The fertile soils, previously used for livestock only, are increasingly used for agriculture. The overpopulation of this area is also due to the resettlement of thousands of people from the centre of Chad following the severe droughts of the 1980s in the Sahel (Planel, 1996).

Resources access and lack of clear regulation

The basic pastoral legal frameworks date from the French colonisation and seem inadequate for this context. The rules of access and use of land are poorly enforced and give more benefit to people with greater bargaining power. With the socio-political unrest in the country, many less qualified authorities have been appointed as heads of the local administrations. The management of local resources is hardly based on legal frameworks, leading to conflicts of interest and land grabbing by those who can skillfully apply the rules for personal interest. Pastoralists compete for diminishing resources in the Lake area. The aim of this competition is mainly for the occupation of good pasture and avoiding water bodies in the transhumance road. There is also a competition with local settled breeders and mobile pastoralists for pasture.

The multiple taxes and fines

At least nine legal taxes associated directly or indirectly with livestock and livestock product trade were enumerated (exportation tax, internal tax for transport, market tax, slaughtering tax, tax of meat certification, tax of skin and leather certification, tax of raw meat exportation, tax of buccaneer meat). These taxes were to be paid to traditional chiefs and representatives of the mobile pastoralists.

Normally, there is no tax outside of animal trade, since abolition of the livestock tax. However, local authorities (military forces, paramilitary and administrative authorities) skilfully found ways to extract money. All new laws were interpreted locally, for example the order of the ministry of environment to prohibit wood cutting was extended to activities of livestock keepers (fencing) and farmers (clearing of fields). A local expression says "breeders live on animals and authorities on breeders".

The obligatory mediators

Mobile pastoralists cannot sell and buy an animal without a guarantor called "wekil". This person should come from the same administrative area as the mobile pastoralists and should

be appointed by the traditional chief and confirmed by the local administrative authority. The role of this guarantor is to protect pastoralists in case of litigation. However, this mediator role was often exaggerated. "wekils" negotiated between pastoralists and traders for personal profit, and pastoralists were obliged to accept because they could not sell without the approval of the "wekil" imposed by traditional and administrative power.

Illicit credit

Among sedentary agricultural communities there is a credit system called "salam". One can borrow from the traders during the periods of supply shortfall and pay back in kind after the cereal harvest at exaggerated prices fixed during the negotiation. Another system, called "riba" is the borrowing of money or cereals to be paid back with interest rates as high as 50%.

Good accessibility to cities

On one hand, it is an advantage for producers to easily sell their products but on the other hand it increases living costs in rural areas. The south shore of the Lake Chad is the most accessible zone for resources in Chad with a tarred road between N'Djamena, Guité and Karal. Other causes for increased living costs were the food stocks kept by the National Office for Food Security (ONASA) and uncontrolled speculation by traders.

Local food and social behaviours

Although mobile pastoralists are near to Lake Chad, many of them do not consume certain highly nutritious foods. For example, the consumption of potatoes and beans is considered shameful among the Dazagara community coming from Kanem and the Bahr-el-Gazal regions. This is due to their pastoral way of life with feeding habits influenced largely based on meat and dairy products. However, there are no food taboos due to religion except the consumption of fresh blood.

Overview

The food security in Sahel region was threatened by structural as well as circumstantial factors. Grégorie (2005) stated that socio-cultural and biophysical factors negatively affect food security. The major strategy of pastoralists is focused on the seasonal variation animal and food stock prices (Figure 3). Animals were sold during favourable periods when animals took weight and prices were good. These periods correspond also to the harvesting period of crops with abundant availability of cereals at low prices. Hasen (2002) and Oram (1989) mention the importance of seasonal variation on the human activities and particularly on the agricultural production.

In addition, the mobile pastoralist's instruction level is very low. The national level of alphabetization was at 27% among women in 2005 (Bandoumal et al., 2005). The average age of interviewed women was low because the target population of this study based on the mother/children couple, whose nutritional status is reported elsewhere (Bechir et al. 2010). This explains why we have more young women at child bearing age and the relatively low number of children per woman.

Market accessibility was relatively good in this region, and camps and villages were not far from the weekly markets. This is not the case for all pastoral communities, for example, in Timbuktu, Mali where a cereal bank was requested because of the lack of a market (Bassirou Bonfoh personal communication). Market and transhumance management were favoured by the exchange of information with traditional and new information and communication technologies (ICT). ICTs, such as mobile phones, play an important role for mobile pastoralists in Chad in the exchange of information on prices and availability of pastures. This tool was also noted by Corradi (2009) in Mauritania.

The correlation between the average price of Zebu and the average price of 100 kg of corn shows that sale money from a single Zebu could provide 11.4 (100 kg) bags of corn. This covers the needs of a family of six persons for one year at a level of 191 kg/person/year, which is higher than the national requirement, fixed at 150kg/person/year (Fews Net et USAID, 2008). The sale of one small ruminant is equivalent to 1.2 (100kg) bags of corn. More than half of nomadic women (56%) manage more than seven dairy zebus, which is enough to cover the needs of a family of 6 persons in Sahel (Pagot, 1985). These amounts are applicable when mentioned products are self-consumed. Usually, mobile pastoralist and rural sedentary producers sell their produce and pay more for other goods rather than investing in their own wellbeing. Despite their relatively high purchase power, children of mobile pastoralists and to a lesser extend sedentary communities suffer from severe malnutrition with levels of acute global malnutrition ranging from 10-18%, depending on seasonal variation (Bechir et al. 2010).

A pastoral legal framework (pastoral code) was adopted in 1955 during colonial times (Law n° 4, 31 October 1959). However, the overlapping basic and customary law (Law n°24 by 22 July 1967) and the instability of Chad did not allow for strong institutions to be put in place (Yosko, 1999; Fokou et al., 2008). Abuse by unqualified administrative and military authorities combined with numerous rebellions since 1979 contributed to maintain mobile pastoralists and rural sedentary communities in a weak bargaining position. Authorities contribute to impoverish local communities and increase their vulnerable status. Fokou (2008) showed that an important fraction of the income of pastoralists in the Lake Chad region is taken off by administrative, military and paramilitary authorities constantly changing regulations to serve personal interests.

In the same time, the area of Lake Chad serving as a unique resource pocket for the whole Sahel region is subjected to high competition between breeders, cultivators, fishermen and safari hunters. The usable area is reduced every year while human and animal populations continue to grow. Nevertheless, mobile pastoralism remains an ecologically and economically sustainable way of managing fragile natural resources in the Sahel area. The Lake Chad Basin Commission (LCBC) and the Development Society of Lake Chad (SODELAC) attempt to mitigate the pressure on the land within the prevailing institutional setting.

The key determinants of malnutrition noted in this study are summarized by a conceptual diagram based on the access framework by Obrist et al. (2007) which includes the role of livelihood assets (Figure 8). The non-application of pastoral legal frameworks (pastoral code) and the lack of strong institutions contribute to maintain pastoral communities in a vulnerable position and limit their access to resources and social services. These factors affect malnutrition through food insecurity, contributing to micronutrient deficiencies

(Zinsstag et al. 2002). Lack of potable water causes parasitic infections (Bechir et al. 2012), which is even worsening the effects of malnutrition and increasing morbidity and mortality. This qualitative social-ecological systems analysis (Zinsstag et al. 2010) reflects the importance of livelihood assets presented by Obrist (2007). The analysis of livelihood assets adapted to the nomadic context, as presented in this paper, allows identifying key assets related to social vulnerability in nomadic areas. These assets include (1) human capital: developing local knowledge and education; (2) social capital: strengthening community networks and locally adapted services (Schelling et al. 2007); (3) natural capital: valorising sustainable pastoral resource use and access to land and water; (4) physical capital: implementing locally adapted infrastructure, logistics and tools; (5) financial capital: developing livestock banks and improve market functioning. (Figure 8)

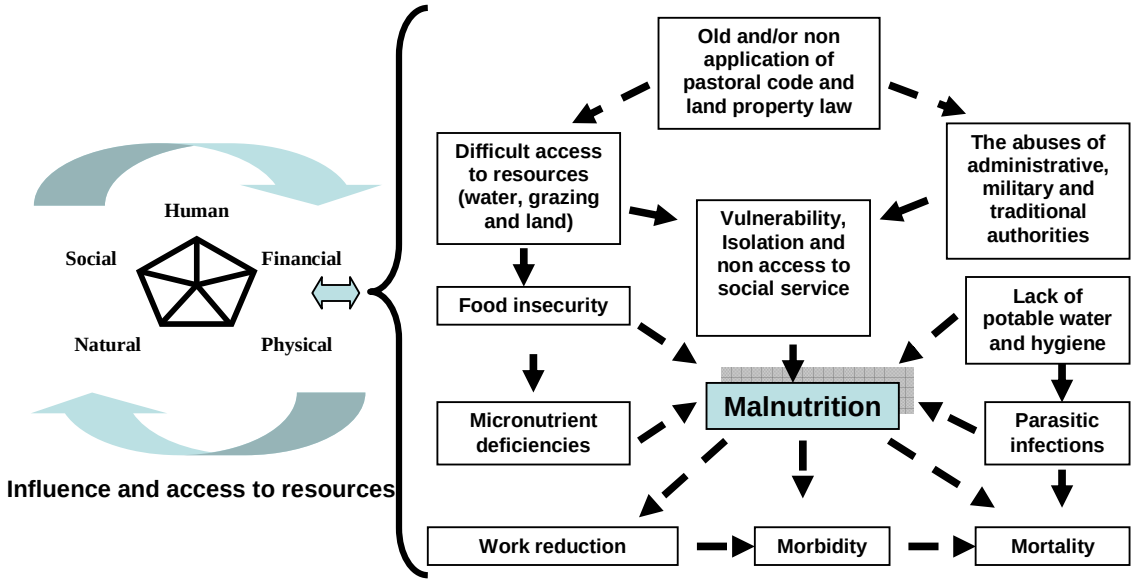

Figure 8: Key determinants of malnutrition in nomadic area in Chad

Facing this vulnerability, pastoralist communities develops resilience to reduce this burden.

The Table 2 shows the main vulnerability and resilience among pastoralist.

Table2: concept of vulnerability and resilience

Concept of vulnerability and resilience	
Vulnerability	**Resilience**
- Rapid population growth and lack of social service access; - Climate change and draught ; - Lack of best pasture and weak of milk production - Risk factor link to transhumance through different region; - Lack of formal education and ignorance of low; - Maladjustment of colonial pastoral code and fundamental low ; - Insecurity and abuse of authority	- Mobility (access to pasture) ; - Selection of rustic animal for livestock; - Diversification of animal (mixt cattle, sheep and goad) and activities; - Handicraft activities of women; - Increase number of milking animal to improve the weak production; - Good empiric knowledge; - Mastering pastoral management ; - Selling animal in rainy season and storage food

CONCLUSION

Mobile pastoralist communities in the Lake Chad area have developed adapted strategies to cope with difficult conditions in the Sahel. They generally choose the end of the wet season, when the animals are heaviest, to sell their off take. In this period, livestock prices are better and cereals are cheaper. They stock their cereal in the neighbouring villages during this time of food and finance availability. Even if food security in these areas is acceptable, underlying causes decrease the health and wellbeing benefits nomadic pastoralists and rural sedentary populations receive from their resources. These causes include illicit credits, market intermediaries, multiple taxes and fines and the lack of institutional frameworks to protect their assets and provide legal security. However, this situation could be changed. A new institutional setup through which abuses could be systematically reported and wrongdoers clearly sanctioned is urgently needed. This would likely contribute to sustainable and real development and secure the health and wellbeing of vulnerable mobile pastoralist and sedentary communities in the study area and in similar contexts.

The general recommendations are:

- to revise the pastoral and basic legal frameworks and codes taking into account the new context and the particularity of the natural resource area at Lake Chad;
- to introduce agricultural credit systems to counter informal and illicit credit systems;
- to encourage the profitability of agriculture by putting in place modern systems and methods;
- to promote culling of animals to reduce the grazing pressure, accompanied with sensitization for a change on behavior in livestock keeping;
- to regulate access to pastoral resources in Lake Chad with a flexible calendar dependent on rain variation and transhumance movements;
- to create pastoral facilities (wells, transhumance routes) in the areas of origin of mobile pastoralists from Kanem and Bar-el-Ghazal and improve their access to basic social services;

- to abolish the guarantor representation which maintains pastoralists under unnecessary tutelage

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8

Spirulina: An Effective Dietary Supplement for Malnourished Children in Development Country

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ABSTRACT

A food ways of improving nutritional status is the use of spirulina. It is a micro algae rich in protein, vitamins, minerals and trace elements. The food is abundant in the lakes of Chad. However, many consumers are unaware of the existence and importance of this commodity used in many countries as a solution to the above child malnutrition. Food security is an important determinant in the development process. In countries Roads Development (PVD), millions of people suffer from chronic malnutrition and micronutrient deficiencies are a real public health problem with significant economic consequences. Nutrient deficiency in food rations causes serious diseases. In many developing countries, child malnutrition is evolving very differently from one region to another. The results of nutritional surveys are particularly disturbing for very young children. Given these conditions, the improvement of the nutritional situation could only be achieved by strategies to combat malnutrition.

KEYWORDS: Spirulina, Production, Consumption, Malnutrition, Chad

INTRODUCTION

Spirulina is a small aquatic being (0.3 mm long), old as the world, whose scientific name is "*Arthrospira platensis*" (not to be confused with the marine cyanobacterium scientifically called *Spirulina subsalsa*), which lives of photosynthesis like plants and thriving naturally in saline and alkaline lakes warm regions of the globe (Jourdan, 2006). Spirulina, already described by Wittrock and Nordstedt in 1844, was really rediscovered in 1940 in Chad by a French botanist named Dangeard (Elyah, 2003). In Chad, common name of spirulina in Chad is "Dihe". This algae has long been used by the "Kanembous" tribe of Chad. These are "Kanembou" women who make the harvesting and drying of spirulina. The introduction of the mass culture of microalgae at the end of the 1950, spirulina has resurgence in popularity for human consumption (Elyah, 2003). The *Spirulina platensis* grows naturally in areas of Lake Chad and Kanem. The oasis found there, called ouaddis have the distinction of having in the center water with high concentration of natron in which develops spirulina. The Spirulina grows preferentially in warm water alkaline and rich in nitrogen and phosphorus nutrients. Commonly, it is found in brackish and saline lakes in tropical and semi-tropical (Castenholz et al., 2001 cited by Charpy et *al*. 2008). Its thermophilic character and important light requirements limit its range to an intertropical band between approximately 35 ° north and 35 ° south latitude. Its high ecological plasticity allows the back to nature in both alkaline lakes in Africa (Chad, Ethiopia, Tunisia), Latin America (Mexico, Peru), South Asia (India, Sri Lanka, Thailand). However, it is much less abundant in North America and

Europe (Charpy et *al*., 2008). Spirulina has more interesting qualities for food and health for both men and animals. It's now grown in large factories in the USA, India, China, Thailand, etc. For example, kwarshiorkor children can be recovered by feeding them spirulina per day for one month (Jourdan, 2006).

HISTORY OF SPIRULINA

The microscopic blue-green algae are the first plants to have appeared on earth there are more than three billion years ago. It is believed that the algae, during another billion years, until the appearance of the first plant, ensured the photosynthesis on Earth and acting on the phenomenon of air regulation.

Spirulina: from its discovery to the present day: Spirulina was first discovered in 1492 (logbook Columbus) in Mexico where the Aztecs consumed as dried small cakes called green "Tecuitlatl" (Delpeuch et *al*., 1975). Spirulina is also called "Dihe" which used as a sauce or cake is also used long ago by "Kanembou" of Chad.

In 1939 Mrs. Creach, pharmacist in Bordeaux found spirulina cakes dried in a market of Massakory in Chad. These were used by people sick or after childbirth and also by injuries following hunting or wars between rival tribes. Spirulina found on the market under the name of "Dihe". It brings back some samples in France for analysis and identification. However given the time (2nd World War), the research was stopped after the death of Mrs. Creach.

In 1940 a French algologist identified spirulina and mentions for the first time its food use (Dangeard, 1940). It is mainly from 1946 that scientists began to discover its remarkable properties of spirulina. From the 1960, publications on spirulina begin to appear. And a Belgian botanist J. Leonard, in a trans-Saharan expedition gave a sample of a plant-produced his colleague P. Confederate who identified the substance as *Spirulina platensis* (Leonard, 1966). In his thesis F. Busson presents his research on spirulina in 1971 (Busson, 1971).

Moreover, Hubert Durand Chastel and David Maurice society Sosa Texcoco, producer carbonates extracted from Lakes Aztec Mexico, and Genevieve Clement of French Petroleum Institute discovered that annoying microalgae Lake Texcoco were really excellent food of the Aztecs. They began commercial production in 1976, and since the industry Spirulina has spread around the world and increased by a few hundred pounds for a production estimated in 2001 to approximately 5000 tonnes (Waterlow et *al*., 1960) and 8000 tons (Charpy et *al*., 2008) in 2008.

In 1959, Max-Yves Brandilly (anthropologist) published in "Science and future" an article on the dried cakes. In the early on 1960, the microbiologist Hiroshi Nakamura and Dr. Christopher Hills pioneered research on spirulina, observing its benefits themselves. Dr. Hills, founder of "University of the Trees" in California, President of the Microalgae International Union, has continued to promote the knowledge and consumption of that food.

On 1965, the Belgian botanist J. Leonard who participated in a Belgian-French Saharan expedition made state in Chad to the presence of algal blooms in the wadis and the sale of green blue cakes, from these algae, in the traditional markets of Fort Lamy (now N'Djamena

). Microscopic and chemical analysis of samples of cakes by Leonard and Compere indicated that they were formed almost exclusively of the cyanobacterium Arthrospira (called Spirulina at that time) and revealed the high protein content and the nutrient richness content of this biological food. In 1970 Ripley Fox broadcasts Spirulina in Africa. In 1984 after Mexico (Lake Tex coconut) and Africa (Lake Chad), China (Cheng Lake hated) produces natural and pure spirulina. In 1996 the first international symposium of spirulina was held in Cheng hated Lake, Yunnan, China. In 1999-2000, The University Center of Biotechnology Algale (Cubia) of Liege (Belgium) and Tractebel Engineering Department Consult (Brussels, Belgium) conducted several missions in Chad as part of a prefeasibility study for the development of spirulina production. This study aimed to evaluate the possibilities to optimize the artisanal and traditional use of spirulina in Chad and to develop industrial production. In 2003 there was the birth of the Professional Certificate of local initiative specialty "Traditional production of spirulina humanitarian" written and validated by an institution of the Ministry of Agriculture and Fisheries, after a consensus of professional representatives in France (May 2003) and the Symposium of Microbial Ecology "Carry le Rouet" (25-28 May 2003). In 2002 the holding of a conference in Mialet production on artisanal spirulina (26-28 June 2002). The first objective is the establishment of an experiment that will produce spirulina under greenhouse in littoral areas. Other seminars were held in the Embiez Islands (2004), Niger (2006), Togo (2008) and Cameroon (2013).

ELEMENTS OF THE BIOLOGY OF SPIRULINA

Origins

Spirulina is an aquatic organism (0.3 mm long), in a spiral shape and green-blue color. Appeared on earth there are over 3.5 million years, it is as old as the world. These microorganisms helped transform the original atmosphere of our planet by producing, through photosynthesis, producing the oxygen necessary for the evolution of all other forms of life. The earliest forms of life on Earth were bacteria, which already existed 3.4 billion years ago, and then the cyanobacteria or blue-green algae appeared: Spirulina was born. She was able to adapt to all the physico-chemical conditions for the time immemorial until today. These first plant cells transformed carbon dioxide into organic matter and cleared the oxygen. Often classified as algae, it is not one itself. It is a food, a microorganism which belongs to the class Cyanophyceae, that is to say a form of primitive life combining features of algae and bacteria, but considered different and both commonly called blue-green algae or mucilaginous algae (Anonymous, 2009).

Taxonomy

Spirulina belongs to the order of the Nostocales (Oscillatoriales), family of the Oscillatoriaceae, genus Oscillatoria and under genus Spirulina or Arthrospira (Charpy et *al*., 2008).Spirulina is a cyanobacteria (formerly designed by the term *blue-green algae* and then algae). It therefore belongs to the domain of bacteria and ranks among gram-negative bacteria. Cyanobacteria form the bulk of bacteria capable of photosynthesis to produce oxygen and may be unicellular or multicellular.

Description

There are two main species: the species *Spirulina platensis*, a native of Kanem (Chad) and the species *Spirulina geitleri* or maxima, originating from Mexico (Delpeuch et *al*., 1975.). Spirulina is in the form of multicellular filaments called trichomes, unbranched and may under certain conditions be surrounded by a mucilaginous sheath. Its main features are its blue-green color and its helical shape, which is constant and which it takes its name. Its average length is 250µ when she turns 75 (there may be few turns 1-3 or much more than 100). The diameter of the coiled filament is about 10µ (sometimes the turns in the center of the trichome are closer to the ends and the general shape is that of a pellet, with a smaller diameter of the turns to the ends and center). The cell diameter can range from 3.5 to 11µ. However the spirulina has great flexibility related to the conditions of the culture medium (Clement, 1975).

Electron microscopy (Figure 1) .has allowed a better understanding of the structure and functioning of cells of cyanobacteria. Indeed, the organization of this cell is the same as that of prokaryotes: cell wall is composed of a membrane consisting of four thin layers mucopolymeres and polysaccharides without cellulose; there is a lack of core despite the presence of nucleic acids not surrounded by a membrane.

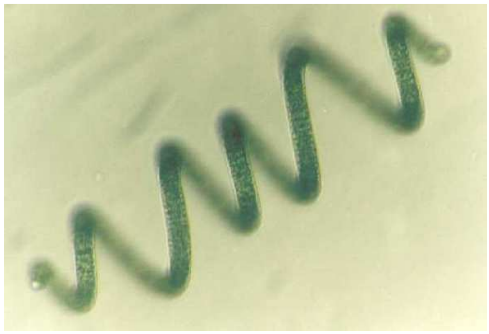

Figure 1: Spirulina seen under the microscope. Source : (ASMH, 2010)

Life Cycle

The filament of Spirulina at maturity form specialized cells called necridies. They differ from other cells by their biconcave appearance and are treated as separate disks. From these, the trichome fragments to give new filaments of 2-4 cells called hormogonia. The hormogonia will grow in length by binary division (each cell will give two cells by fission) and take the typical helical. Under experimental conditions, the generation time (from one generation) Maximum of Spirulina is about 7 hours (Zarrouk, 1966).

Optimum conditions for the growth of Spirulina

To develop, Spirulina needs simple minerals such as water, minerals, carbon dioxide and oxygen it draws directly in the environment while using sunlight as a source of energy through the pigment system. This mode of biomass system is autotrophic picture. Spirulina grows in natural environments characterized by brackish water, hot, alkaline and natron of the tropics. As a general rule the phosphates, carbonates, nitrites and iron elements are

limiting phytoplankton production in aquatic environments. In natural deposits, these are provided by watersheds. Spirulina grows in warm water (28-40 ° C) and boasting a high luminous intensity. The wind plays an important role by creating an agitation which promotes a homogeneous dispersion of Spirulina in the middle, and therefore its exposure to light. In the wild, when conditions are optimal, the Spirulina can grow in large quantities and then compete with other organizations. During blooms, the consumption of carbonates and bicarbonates increases the pH thereby limiting the growth of other microorganisms (Elyah, 2003).

Ecology of spirulina

The development of spirulina, it is in the wild or in a controlled culture requires an environment comprising water, an area of suitable temperature, light to provide energy for photosynthesis, nutrient balance acid base and favorable pH. Spirulina grows in sodic lakes all continents. It can withstand very high salt concentrations. Its growth is optimal at concentrations of 22 to 60 g / l of salt (Iltis, 1974). The optimum pH of a thriving culture is between 9 and 11. At high pH spirulina are the only practically pushing themselves as resistant as alkaline medium (Bussson, 1971). The optimum temperature for growth of Spirulina is included between 32°C and 40°C, with a range of 18 ° to 50 ° C. The limiting factor for growth is the daytime temperature, which must not drop below 20 ° C (Ripley, 1983).

Nutritional

The protein content of spirulina is high (Table 1). It represents 10-11% of the wet weight, 60 to 70% of its dry matter (Clement, 1975). This percentage is much higher than that of fish (25%), soybeans (35%), powdered milk (35%) and cereals (14%) (Henrikson, 1994). Spirulina is rich in nitrogen content and contains twice as soybean, three times more than the meat or fish. However, this wealth into perspective given the small amount of spirulina dietary supplement used (<10g per day) (Charpy et *al*., 2008).

Table 1: Average percentage of amino acids in *Spirulina platensis* by different authors and S*pirulina maxima* by Borowitzka, Borowitzka (1988).

Amino acid	Jacquet 1974	Clément 1975 b	Fox 1999	Borowitzka 1988
Essential amino acids (%)				
Isoleucine	5,60	6,40	5,98	5,70
Leucine	8,00	9,00	8,71	8,70
Lysine	4,20	4,80	5,28	5,10
Méthionine	2,25	2,60	2,85	2,60
Phénylalanine	4,40	4,60	5,09	5,00
Thréonine	4,70	5,50	5,58	5,40
Tryptophane	1,00	1,60	1,48	1,50
Valine	5,70	6,90	7,72	7,50
Nonessential amino acids (%)				
Alanine	7,25	7,90	8,24	7,90
Arginine	6,60	6,70	7,92	7,60
Acide aspartique	9,30	9,20	9,50	9,10
Cystéine	0,95	0,90	0,93	0,90
Acide glutamique	NC	12,90	13,20	12,70
Glycine	4,80	5,00	5,07	4,80
Histidine	1,60	1,60	1,50	1,50
Proline	3,60	3,90	4,32	4,10

Sérine	5,00	5,60	5,46	5,30
Tyrosine	4,30	4,90	NC	4,60

The total lipid composition (Figure 2) is characterized by a good balance between saturated fatty acids and polyunsaturated fatty acids (PUFA). It is divided into two fractions: a saponifiable fraction "or fatty acids" (83%) and an unsaponifiable fraction (17%).

Figure 2: Drying traditional "Dihe" (FAO 2009)

Carbohydrates represent 13.6 to 25% of the dry matter of spirulina (Falquet and Hurni, 2006; Quillet, 1975). In *S. platensi* As in *S. maxim*, values of 4.2 to 6% are reported on total nucleic acids in the dry matter (Santillan, 1974; AFAA, 1982 cited by Falquet and Hurni, 2006.).

Note also the exceptional level of vitamin B12 (cobalamin) wihch is by far the most difficult to obtain in a meatless diet vitamin because no power plant contains. Determined according to the old standard method, spirulina is four times richer than raw liver, long given as the best source (Falquet and Hurni, 2006).

Table 2: Typical percentage composition of the main fatty acids of three species of Spirulina by Pascaud et al. 1(993)

Fatty acids	***Spirulina pacifica***	***Spirulina maxima***	***Spirulina platensis***
Palmitic (16 :0)	44,2	63,0	25,8
Palmitoleic (16 :1) omega-6	4,4	2,0	3,8
Stéaric (18 :0)	Traces	1,0	1,7
Oleic (18 :1) omega-6	0,4	4,0	16,6
Linoleic (18 :2) omega-6	24,3	13,0	40,1
Gamma-linoleic (18 :3) omga-6	22,1	13,0	40,1
Alpha-linoleic (18 :3) oméga-3	Traces	Traces	Traces

Among the fat-soluble vitamins, there is a very high β-carotene content. This provitamin A represent 80% of total carotenoids (Pierlovisi, 2007), the remainder consisting mainly of xanthophylls, cryptoxanthin, and echinenone, zeaxanthin and lutein. Vitamin E has antioxidant properties to unsaturated fatty acids and keeps well after drying of Spirulina. It was noted in the levels of vitamin E content of Spirulina ranging from 13 (Gomez-Coronado et *al*., 2004) to 120µg / g (Vincenzini et *al*., 1979). This highly variable level of vitamins (Table 3) is probably related to different methods of dosing and different qualities of Spirulina.

Table 3: Vitamin content in µg/g of dry matter of Spirulina by Falquet and Hurni (2006) completed with other references to Vitamin E

Vitamins	Content
Water soluble vitamins	
B1 (thiamine)	35-50
B2 (riboflavine)	30-46
B3 (niacine)	130
B5 (pantothénate)	4,6-25
B6 (pyridoxine)	5-8
B8 (biotine)	00,5
B9 (folate)	0,5
B12 (cobalamine)	0,10-0,34°
C (acide ascorbique)	Traces
Fat-soluble vitamins	
Provitamine A (β-carotène)	700-1700
Cryptoxanthine	100
Vitamine E (alpha-tocophérol)	120°° ; 50-190°°° ; 13°°°°

° Excluding nickname vitamin B12 ; °° Vincenzini et al. (1979) ; °°° Falquet et Hurni (2006) ; °°°° Gomez-coronado et al. (2004)

Table 4: Mineral composition of Spirulina grown in µg / g of the dry matter by Falquet & Hurni (2006)

Minerals	Content	Doses required ° (mg/jour)
Calcium	1300-14000	1200
Phosphorus	6700-9000	1000
Magnesium	2000-2900	250-350
Iron	580-1800	18
Zinc	21-40	15
Copper	8-10	1,5-3
Chrome	2,8	0,5-2
Manganese	25-37	5
Sodium	4500	500
Potassium	6400-15400	3500

° Dose required for adult

Compared to its mineral composition (Figure 4), a large variability was observed in the levels. It is explained by the fact that they relate to the natural environment and Spirulina cultivated ones. Variability in controlled cultures is much lower. In addition, it is possible to increase the mineral content of cultured organisms (Falquet and Hurni, 2006). Natural Spirulina rarely have iron contents exceeding 500mg/kg although higher than 1000 mg / kg values have been found (Campanella et *al*., 1999). Spirulina culture can be enriched in iron. Zinc is considered as a major in the fight against micronutrient malnutrition (Gibson, 2005). Spirulina cultivated generally contains only traces of zinc (21-40µg / g). These levels are insufficient for Spirulina is considered a good source of Zinc. Indeed, the need for Zinc a child from 6 months to 3 years is estimated 3103-5103 mg. If the entire Zinc was bioavailable dose of 10 g of Spirulina would cover 5 to 10% of these needs. However spirulina can be

enriched (Cogne et al., 2002). A Spirulina Biorigin the Azina titrerait 6000µg Zn / g. Antenna Technology developed a simple protocol to enrich Spirulina Zinc (Falquet and Hurni, 2006). Mg is an important element for health and its deficiency is common in malnourished children (Briend, 1998). Spirulina also contains magnesium, potassium, iodine, and pigments such as chlorophyll.

Figure 3 : Cakes: traditional form of Spirulina **(**FAO 2009)

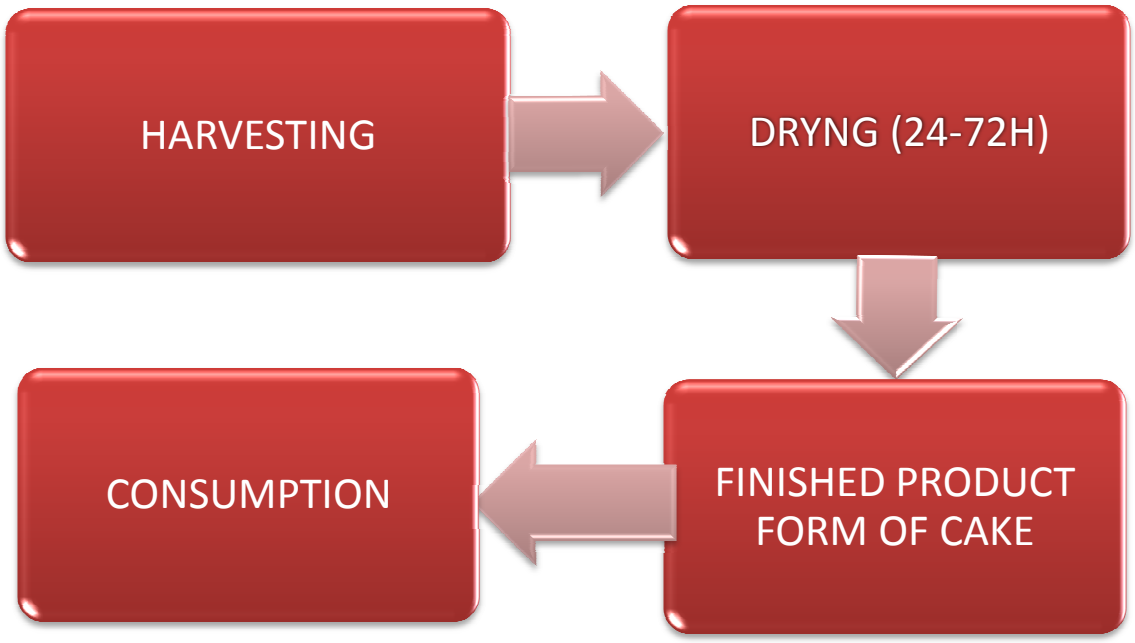

Figure 4: Diagram of artisanal production of siprulina "Dihe"

Harvesting, drying and packaging

It is better to harvest in the morning because the content of spirulina protein is generally higher than in the evening, but also for other reasons: excessive heat then need to dry the crop as soon as possible (especially if Solar drying if the weather is not assured in the afternoon). Under good conditions it is possible to harvest each day sixth to 1/3 of the culture. Culture was filtered through two devices, generally superposed. The first consists of a fine mesh (300µm mesh around empty) holding Spirulina. Wet biomass is pressed. Fresh

Spirulina thus obtained can be consumed directly, or dried for storage. Drying is the only sure way to preserve and distribute spirulina without cold chain. Industry spirulina is typically dried by "spray" (spray-drying) in a stream of combustion gases at very high temperatures but for a very short time. For this, the filaments must first be pulped to break their membrane, which is actually the juice of crushed Spirulina is dried. Unless the drying gas is very low in oxygen, spray drying is likely to alter the product. Biomass is extruded spaghetti so that you can more easily dry. It is dried in dryers, gas or electric. Standard water content of Spirulina is dried below 10%. Spirulina usually devoted to marketing includes 7% water. Drying in an oven to 60 °C does not appear to significantly alter the properties of Spirulina. Spirulina is then dry milled powder form or flake form and stored in an airtight container away from moisture and light. Spirulina can be packaged in bags, boxes or bottles in the form of twigs, powder, capsules and tablets.

PRODUCTION OF SPIRULINA IN CHAD

Production craft

Women enter the water and collect the "Dihe" using cups, buckets and basins. After the the harvest "Dihe" is directly transferred into bowls sands appointed for the occasion, which over time absorb water. After two to three days of drying (Figure 2), the product whose thickness can vary from 5 to 8 cm becomes dry and the final product is obtained in the form of cakes (Figure 3).

Traditional production improved

Women enter the water and collect the "Dihe" using cups and plastic buckets. The "Dihe" thus collected was transferred to a 5 mm mesh screen that removes large and medium-sized impurities. The product is then recovered in a second mesh screen which retains 600 µm that retains the spirulina and let's move a large part of the water. After this step, the solution is recovered and then pressed into a fabric by hand in order to remove much of the water is obtained in the form of "Dihe" (form of a ball). The latter is in an extruder. After extrusion the "Dihe" is obtained in the form of cake or spaghetti. Spaghetti and cakes are spread on trays and then dried in the solar dryers whose upper part is translucent sheets. All four sides of the dryer with wire (Figure 5) to avoid contamination by insects. The product becomes dry solar dryer after 4 to 5 hours (Figure 6).

Figure 5: Improved Drying Technology

Source : FAO (2009).

Figure 6: Spirulina improved in sachets

Source (Aguid, 2010)

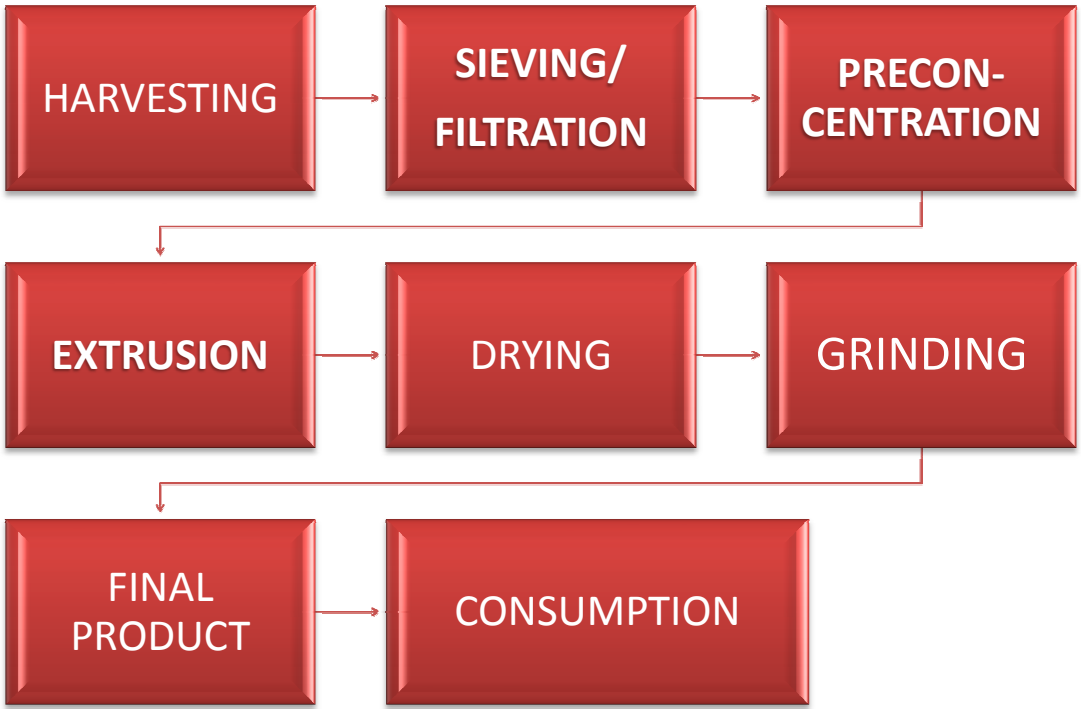

Figure 7 : Improved process of the Spirulina diagram "Dihe"

Latest research Studies

Tests on malnourished children

In Dakar Senegal, Experimentation with blue-green algae (spirulina) was conducted on 59 children with protein-energy malnutrition has given a remarkable cure rate of 88.14%. The administration of spirulina is easy. The product is also well accepted and well tolerated.. The anthropometric evolution (gain of 50 g / day), clinical and laboratory (175% for the prealbumin), demonstrates the remarkable nutritional qualities of spirulina (Sall et al., 1999a). If its cost is competitive, spirulina could be of great interest in the recovery and prevention of protein-energy malnutrition.

Another study entitled "*Evaluation of the efficacy of spirulina supplementation of the usual diet of children suffering from severe malnutrition (About 56 cases)*" was conducted by Habou (2003) in Niger. The work involved a sample of 56 patients, severe malnourished aged form 6 to 24 months these all selected children to the hazards were followed for 14 days. 10 g of spirulina daily was added to the usual diet of malnourished children. The results of this study show a rapid clinical improvement from the fourth day with a quasi complete amendment to the 7th day. As for biological signs, they achieved good progress overall with growth rates of about 30% for serum protein and albumin, 60% for the prealbumin, 4.5% for hemoglobin and a regression leukocytosis of 19.27%.

A thesis entitled "*Comparative study of two diets in the treatment of malnourished children in Bangui: Spirulina-sardine versus corn-soy*" was defended in 2005 In Bangui (Central Africa Republic) (Dupire, 2011). The randomized study of nine set focused on two groups of 75

malnourished children. (Especially on kwashiorkor). The first group received 5 g of spirulina daily associated with sardine oil, while the other group received 150 g of corn-soya blend. For Spirulina sardine group, the recovery time was 38.6 days with a mean weight gain of 12.3 g/kg/day, against 50 days and 7.1 g/kg/day for the group corn-soya with a significant difference. The regression of clinical signs in the first week of treatment was in favor of Spirulina sardine group. The effectiveness of care observed proceeds in much of the nutrition education to parents of malnourished children. The study also concludes that in terms of sustainability and income generation, spirulina fish is associated with more favorable and it would be wise to promote their production and availability in the market at a competitive price.

Another study among people living with HIV still in Bangui, CAR showed that serum protein is significantly higher in patients receiving spirulina with lower parallel increase in creatinine suggesting that protein intake by Spirulina is significant (Yamani et al., 2009).

In Chad, A study entitled "*Testing of nutritional rehabilitation of children from 6 to 59 months spirulina versus Ready-To-Use Therapeutic Food*" showed that the administration of spirulina was easy, the product was also accepted and well tolerated by children (Nawo, 2013). The study was randomized, controlled and double blind. The average duration of treatment was 29.36 days for spirulina and 37.05 days spirulina for other foods. It focused 300 severely malnourished children aged from 6 to 59 months divided into two groups. Spirulina was administered at a rate of 10g per day mixed with the family meal in the first group and the second group received food (Ready-To-Use Therapeutic Food) depending on the clinical state presented by the patient.The results of this study showed a rapid amendment clinics an average of 4 days and an average of 10 days in their disappearance in both signs. As for biological signs, they achieved good progress overall with growth rates of 21.66% (significant increase) for hemoglobin and a decrease of leukocytosis of 41.27% in the first group (using spirulina) against 15.51% and 31.81% in the other group (food without consuming spirulina). The average weight of children grew by 16.34% to an average of 29.16 days with spirulina against an increase of 17.80% in an average of 37.05 days on the side of other foods.The study concluded that spirulina combined with other foods is effective in fighting severe acute malnutrition as well as other ready-to-use therapeutic food.

Many other authors (Sawadogo et al, 2004. Cruchots 2008; Sguera, 2008) mentioned in their work the interest of spirulina for health. According Sall et al. (1999b), the nutritional properties of spirulina are a food source that deserves special attention in our developing countries which is acute problem of food availability. That is why it is desirable to develop the production of spirulina both domestic scale for home consumption at the level of lakes and ponds where can easily grow Spirulina for the commercial component. The development of Spirulina production should be accompanied by the development of processing techniques to artisanal or semi-industrial level, but also the development of recipes enriched with Spirulina to benefit vulnerable groups of our country.

Microbiological quality

Samples "dihe" analyzed in Chad in 2009 showed mixed results (Aguid, 2010). Compared to total bacteria, the extreme value varies from 25 to $3{,}7.10^5$ UFC/ g. The presence of these bacteria could be explained by the binding of local conditions and crop drying. When Wu and Pond (1981), they found 3.10^4-6.10^5 UFC/g in the liquid medium. After harvesting and

drying, spirulina contained only 10^3 to 10^6 of viable microorganisms per gram. This number decreases steadily with storage time mainly due to drying (Wu and Pond, 1981). This number could be explained by the binding of local conditions and crop drying, however, a high number of viable organisms often come from contamination of raw materials, lack of hygiene, of length or temperature at the stage of production storage or combination of these factors (Refaï, 1981). Coliforms were absent in the samples analyzed. It is the same salmonella. Studies on the spirulina produced industrially in the USA and Japan have also demonstrated the complete absence of pathogens such as Salmonella, Shigella and Staphylococcus in their samples (EFS, 1986). The absence of coliforms in samples confirms the good hygienic quality of the raw material (Bourgeois et *al*., 1991).

The study counted 10^3 UFC / g of mold in the analyzes. But lower values were obtained in the USA and Japan. EFS (1986) concluded that spirulina industrially produced contains less than 100 spores per gram of viable fungi. Also, it appears from studies by Vermorrel that the microflora associated with spirulina cultures is usually scarce and non-pathogenic. Yeasts are absent in the samples. Also the high alkalinity of the culture medium is an excellent barrier against most bacterial contamination as well as yeasts (Vermorel et *al*., 1975).

CONSUMPTION

Compared to consumption, it is in the eating habits of “Kanembous” transform the “Dihe” in different kinds of sauces: sauce only “Dihe”, sauce “Dihe” with bean, “Dihe” with dried fish s and “Dihe” sauce with meat. For other types of sauce, beans, dried fish and meat are added to the time of mixing before the final cooking (Sorto, 2003). However, outside the tribe "Kanembous" , many consumers in Chad unaware of this important foodstuff used in many countries as a solution to especially child malnutrition. Also, a recent clinical study conducted in Chad helped administer and test the effectiveness of spirulina in 150 malnourished children 0-59 months. The children received their usual meal mixed with 10 g of spirulina. The study did confirm the effectiveness of spirulina in the treatment of malnourished children. An FAO project has introduced improved techniques for harvesting and drying "Dihe" which is now used in many households. Another project on the National Food Security program is currently being implemented for the certification of spirulina and its inclusion in the national protocol for the management of malnourished children in Chad. It also appears from the studies (Aguid, 2010) that all the “Dihe improved” Chad has acceptable hygienic quality for consumption.

CONCLUSION

Despite the significant resources deployed by the international community and individual States, the problem of malnutrition continues to arise with acuity in ways of developing. Spirulina share his remarkable qualities, ease of culture and safety can be an effective and lasting solution to the problems of malnutrition in addition to various nutrition programs. Artisanal production is actually of safety concern because of poor harvesting practices and drying of Spirulina “Dihe” Chad. However, the pilot project for the development of industry of “Dihe” introduced improved harvesting and drying for a healthy consumption of spirulina techniques. The bacteriological quality of improved “Dihe” is satisfactory and Chadian consumers are beginning to taste.

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9

Pickling of Sea Foods for Commercial Utilization in Developing Countries

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ABSTRACT

Like vegetable pickles, fish and prawn pickle hasalso gained popularity in the recent past.The demand for these types of ready-toserve fishery products is increasing dayby day among the non-vegetarian population in our country and throughout the globe.As the malnutrition affects one in three people globally and it remains one of the mostserious health problems round the globe and Asia contributing 50% of the undernourished people.The Food andAgriculture Organization of the United Nations (FAO) estimates that 22% of India'spopulation is undernourished. By maximising the use of fishery products the threat of malnutrition will be reduced .Value added products like fish pickle and prawn pickle increase the shelf life with less input and are commonly consumed globally which will fulfil the need for consumption of animal protein.

INTRODUCTION

Pickles are found across all cultures. The earliest known examples are cucumbers, that are known to have been pickled sometime around 2030 BC in Mesopotamia, when inhabitants from northern India brought cucumber seeds to the Tigris valley.Pickles are mentioned twice in the Bible, were known to the ancient Egyptians (Cleopatra attributed some of her beauty to pickles), and Aristotlepraised the healing effects of pickled cucumbers. The Romans imported all sorts of foods from the countries they conquered, pickling them for the journey in vinegar, oil, brine and sometimes honey. *Garum* or *Liquamen*, a fermented, salted fish-based condiment was a dietary staple and has been found as far north as the Antonine Wall. Not only Europe but, Asia also has a fine history of pickling. For example, the origins of the noble Korean *kimchi* or *kimchee* (of which more later) has a history that can be traced back up to 3000 years. It probably originated in Chinese pickles which were brought into Korea and were modified to form several types of kimchi of common raw materials to suit the taste of Koreans during the Shilla (654 – 935 AD) and Korea (918 – 1392 AD) dynasties.Back in Europe, a pickle crossover occurred when there was a large increase in food preservation in the 16th Century owing to the arrival of new foods in Europe. Ketchup was an oriental fish brine that arrived via the spice route to Europe and eventually to America, where someone finally added sugar to it. Spices were added to these pickling sauces to make clever recipes. Soon chutneys, relishes, piccalillis, mustards, and ketchups were commonplace. Worcester Sauce was an accident from a forgotten barrel of

special relish. It aged for many years in the basement of the Lea &Perrins Chemist shop.Notable pickle-lovers from history include: Emperors Julius Caesar and Tiberius, King John and Queen Elizabeth I of England, Samuel Pepys, Amerigo Vespucci, George Washington, Thomas Jefferson and Napoleon Bonaparte.The English word 'pickle' derives from the Middle English *pikel*, first recorded around 1400 and meaning 'a spicy sauce or gravy served with meat or fowl'. This is different to, but obviously related to the Middle Dutch source, *pekel*, meaning a solution, such as spiced brine, for preserving and flavouring food.

Pickling of plant and animal foods is a relatively old method of food preservation. It is estimated that the first pickles were produced over 4,000 years ago using cucumbers native to India. The ancient Egyptians and Greeks both have written about the use of pickles for their nutritive value and healing power. Pickles were a common food during the time of the Roman Empire and they soon spread throughout Europe. In America, pickles have always been popular. The first travellers to America kept pickles in large supply because they were nutritious and did not spoil during the long journeys. It is interesting to note that Amerigo Vespucci, America's namesake, was also a pickle salesman. He was the main pickle supplier to many ships. The first large-scale commercial production of pickles did not take place until 1820, when Nicholas Appert began selling pickles in jars. Over the years, the pickle production process has become more automated, however the basic pickling methods have changed very little since the technology was first developed.

While there are many different types of pickles, some characteristics are common to all. In general, pickled cucumbers are crisp vegetables, which can be described as having a strong, biting flavour caused by the vinegar in which they are stored. Different pickle manufacturers normally add spices to give their pickles a unique flavour. Dill-flavoured pickles are perhaps the most common of all pickles. There are also sweet pickles, which are packed with added sugar. These are typically used for making relishes. Kosher pickles were pickles that were approved by the Jewish Orthodox Congregations of America, but the word kosher is now often used to describe any garlic flavoured pickle.

Fish or prawns have very limited shelf life and they need to be processed immediately to preserve them for a longer period. Making pickle is one such easy method. Fish/prawn pickle, if made properly under hygienic conditions adding requisite quantity of salt, spices and preservatives, would have shelf life of around 8 to 10 months. Pickles can be made in any part of the country. This note concentrates on the North-East region as it has ample varieties of fish and the non-vegetarian food is fairly popular in most of the states. Manufacturing process is not very complicated and the capital investment is not much. Hence a new entrant would not find it difficult to venture into this product line.

Like any other vegetable or fruit pickle, fish pickle is also enriches and is becoming amongst non vegetarians. With addition of salt, spices and preservatives its shelf life is generally 8 to 10 months.

How Pickling Works?

Pickling is necessary to counteract the growth of microorganisms that are always present on food. Left unchecked, these can cause spoilage and illness (if eaten). When the acidity of a pickled food is high, harmful bacteria like *Clostridium botulinum* can't grow.

Increasing a foodstuff's acidity is the basis of the pickling process. Acid can either be added, or produced by natural fermentation processes. Fermentation is the anaerobic or partially anaerobic oxidation of carbohydrates by either microorganisms or enzymes (as opposed to putrefaction which is the oxidation of proteins). Fermentation can have both positive and negative effects. When the fermentation occurs in a controlled way, it yields lactic acid which inhibits the growth of undesirable microorganisms. The pickling process is biologically complex (and has been studied in detail for only a few economically-important pickles), but consists in part of the actions of yeasts from the *Saccharomyces* family, especially *S.cerevisiae*, which convert carbohydrates to alcohols and bacteria of the *Lactobacillaceae* family which produce enzymes which oxidise the alcohols to lactic and acetic acid.

There are two basic types of pickling:

- Long, fermentation-based pickling requires a period (up to several weeks) of 'curing' at room temperature. During this period, colours and flavours change. Acid is produced as lactic acid bacteria grow. Most commonly the bacteria are stimulated by the addition of salt, usually in the form of brine.
- Quick, unfermented pickling made by adding acid (eg, vinegar, tamarind juice). It's critical to add enough acid to prevent bacterial growth.

There is some blurring around the edges. For example, salt is often added to quick pickles for flavour, and pickled onions - although 'ready' within a short time - are at their best only when they have had a chance for fermentation to 'mature' them. Also, some pickles are moved from one medium to another in between pickling proper and storage. This includes olives (pickled in lye and salt) and cucumbers (traditionally preserved in brine) which are bottled in a vinegar solution. Most commonly available pickle are;

Indian Pickles

In India, unripe fruits such as mango, Indian gooseberry, unripe tamarind, lemon, etc. are used traditionally. Apart from these various vegetables such as gherkin, bitter gourd, carrots, cauliflower, ginger garlic, onion, jackfruit and citron are also pickled. In most cases, only one vegetable or unripe fruit is used for pickling. But, occasionally, a mix of two or more vegetables or unripe fruits were also made. Generally, pickles made from vegetables and unripe fruits are prepared with utmost care so that they cannot spoil and be preserved all year long. Non vegetarian pickles are also popular. These pickles are made from chicken, fish, prawns and mutton.

Chinese Pickles

In Asian countries such as China, pickles are very popular and have also been prepared for thousands of years. Chinese picklesinclude vegetables such as cabbage, lettuce, yellow tea melon, cucumber, carrots, shallots, etc. These and other vegetables are added with sugar and salt and put in vinegar. Apart from vegetables, eggs particularly duck eggs are stored by applying salt, earth, hay and other ingredients and sealed to mature for about one month. Some pickles include soy sauce for fermentation instead of vinegar and in other varieties condiments such as ginger, garlic, chilli, peppercorn, etc. are also added for a hot and characteristic flavour.

Korean Pickles

In Korea, Kimchi is a common pickle which is made with fermented spicy cabbage and it also includes a wide variety of vegetables including soy beans, fish, oysters and many different ingredients are used. Korean pickling process owes its origins to Chinese, but unlike the common Chinese cabbage pickles, Korean pickling process has its own variations according to the local flavour and available ingredients. Korean pickling process usually involves two types, one in which the ingredients along with spicy chilli pastes are fermented, while the other involves milder varieties pickled in water.

Japanese Pickles

In a similar manner, even the Japanese have their own version of pickles which include ingredients such as ginger, gingko nuts, carrots, eggplants, radish, plum, green apricot, soybean paste along with parboiled vegetables.

Fresh Pickles

On the other hand, instant or fresh pickles known as chutneys are also prepared. These instant chutneys are prepared to last only for a couple of days. There is a wide range of choice in the preparation of instant pickles. Many vegetables, herbs and condiments are used for preparing chutneys.

Chutneys are very similar to sauces and ketchup that are made in the west.

Western Pickles

In the west, pickles are generally made from salted cucumbers, gherkins and various vegetables which are soaked in vinegar. Apart from cucumbers and gherkins, fruits such as peaches, pears and apples are also pickled. Western pickles generally have flavours of sweet, sour and salty. On the other hand, Indian pickles are generally pungent in taste.

Cucumber pickles can be generally divided into

Fermented or crock pickles
Fresh pack or quick process pickles
Refrigerator pickles
Freezer pickles

Each of these pickles is made in different ways. For example, in fermented or crock pickles either vinegar is added or the vegetables are preserved in salt brine for several weeks. Salt resistant bacteria present in the vegetables help in converting the sugars into lactic acid or the acetic acid present in the vinegar accelerates the process of fermentation. This is the reason why most cucumber pickles have sour and salt taste.

Fresh pack or quick pickles on the other hand, are also very popular as they are very easy to prepare and are ready to eat. Fresh or quick pickles are not fermented but, heated vinegar and salt solution is poured onto the vegetables. Fruit pickles and relishes are also prepared in this manner as well.

Refrigerated and frozen pickles are prepared in fermentation process. But, instead of storing them at room temperature, they are stored in refrigerators and freezers. In these frozen pickles, the vegetable slices remain crisp. Other varieties of pickles are ;

Acid-based Pickles

The most common liquid for acid pickling is vinegar. This is an impure, dilute solution of acetic acid, obtained by the fermentation beyond the alcohol stage of fruit (usually grapes, but also apples) or grain (usually barley, malt or rice). Examples of vinegar-based pickles include:

- Pickled onions (silverskin or other, in malt or white vinegar)
- Pickled eggs (as found in some British chip shops)
- Pickled red cabbage (the traditional accompaniment to Lancashire Hotpot)
- Pickled ginger (those mysterious pink slices found alongside sushi)
- Italian pickled vegetables (a crunchy mixture of - usually - onions, carrots, cauliflower and sliced gherkins)
- Rollmop herrings (good for hangovers!)
- Pickled pigs' feet ('soul food' of the American south)
- Pickled sausages (eaten in Poland and the Czech Republic)
- Pickled beetroot (gak!)

Dry-salted Pickles

Salt has two effects when added to fruit or vegetables. Firstly it draws water from them by the process of osmosis. Secondly, the salt in the resultant brine triggers the fermentation process of the lactic bacteria. The resultant fermentation tends to be bacteriologically complex and delivers a particularly rich range of complex flavours. In Europe and North America, the most common dry-cured pickle is *sauerkraut* (or*choucroute*, if you prefer), a pickled, white cabbage.

Dry salted pickled limes and lemons are also popular in many parts of Asia, and are an essential ingredient of Moroccan food. The lemons can be either dry-salted or brined and may be dried afterwards.

In Japan, plums are dry-salted to make *umeboshi* - a mouth-puckering delight often eaten for breakfast. A related product (preserved plums) can be found in some Chinese supermarkets. Dried and with added sugar and liquorice, they are intended as sweets.

Brine-based Pickles

Like dry-salting, brine-pickling works by a combination of osmosis and awakening lactic fermentation. In practice, many pickles which should traditionally be brine-fermented include vinegar at varying proportions in their pickling medium. Gherkins and similar small cucumbers are traditionally pickled in brine along with other flavourings to yield the various species of what Americans know simply as 'pickles' (eg, dill pickles, kosher pickles, Polish pickles).

It seems very likely that the art of cucumber-pickling was carried to Europe by the Jewish Diaspora, since the many brine-pickled vegetables found in Middle Eastern cuisines represent the pinnacle of brine pickling. Almost any vegetable is pickled here, from aubergines to chillies to garlic to okra to green beans. Pickles are an essential accompaniment to any Middle Eastern meal. Of particular note are pickled turnips, which turn a delightfully vivid, almost artificial-looking shade of pink.

Fermentation and Pickles

In addition to the basic process of acidulation, fermentation yields other products which add additional flavours, and this is why properly pickled vegetables taste so much better than just vegetables dunked in acid. Aromatic esters, for example, provide much of the characteristic taste of*sauerkraut*.

However, the acme is surely *kimchi*, a wondrous Korean pickle. This can be made from various vegetables, and can be brine pickled or dry-salted. The most common variety involves Chinese cabbage. Kimchis are generally heavily laced with chillies and sometimes other flavourings (garlic, spring onions, ginger, fish sauce). Microbiology contributes many wonderful flavours to kimchi as well as a whole range of health-giving compounds. Although kimchi is repulsive to many at first tasting, the acquisition of a taste for it will enhance your life. Bejaysus, it's good! Most Koreans eat kimchidaily, and often about three varieties will be served with every meal - including breakfast. It is big business and consequently has been studied extensively: there are whole journals devoted to 'kimchi science'.

Lye Pickling

Lye is an alkali (a mixture of sodium and potassium hydroxides) traditionally obtained by leaching wood ashes. We have to be a little cautious with regard to the role of lye in pickling. Firstly, it does not, being alkali, contribute to the pickling process per se, but to the breaking down of food matter as an aid or adjunct to pickling.

The exception is the noble olive, which has been eaten for thousands of years and is a staple of the Mediterranean and Middle Eastern diets. Olives cannot be eaten in their raw state and require pickling to render them digestible. Before pickling, they require treatment with lye to remove substances which would be toxic to the fermentation bacteria (black olives also require pre-soaking in brine to allow the lye to penetrate). Once pickled, olives are packaged in various forms, with the addition of various herbs and spices, in brine, vinegar, oil or dried and salted.

Turning to the less desirable lye products. Okay, admittedly many Chinese people swear by 'thousand-year-old eggs. These are not, in fact, ancient eggs. They are made by coating duck or chicken eggs in a paste of clay, wood ash, tea, lime and salt, then burying them - separated by rice straw to stop them sticking together - for a mere 100 days or so. The resultant egg, once you have washed off the foul-smelling gunk and peeled and sliced it, has a grey-green yolk and a dark, translucent, bottle green 'white'. They are eaten in wedges with a dipping sauce or with more sensible pickles. Their flavour has been inaccurately described as 'cheese-like'. On putting a piece in one's mouth, one first notices a not too unpleasant egginess, which quickly builds up until one's whole being is permeated by a

powerful, nauseating, tangy, sulphurosity. They are also found in the centre of a peculiar variety of sweet bun. Nevertheless, they have their aficionados.

Lutefisk is another kettle of fish. A Norwegian delicacy, it is a piece of dried, salted cod that has been soaked in a bucket of lye for several days. The lye breaks down the fish to achieve a gelatinous consistency. To eat lutefisk is a badge of national pride amongst Norwegians - but even they do not pretend that one has to actually *like* it! Lutefisk is traditionally accompanied by mashed potatoes, a species of pease pudding, grease and bacon. More importantly, *akvavit* is deemed essential to the consumption of lutefisk. One must drink in preparation. There is also *surstroming*- a Swedish pickled, canned herring - dealt with in much the same way, but with tiny new potatoes rather than mash. Still, it could be worse. In Iceland they bury sharks for several months before eating them.

Pickles in Sugar

Fruits are sometimes first pickled (usually in vinegar) before being stored in a syrup or honey. Pickled peaches are a traditional American dish. Alternately, a sweet-sour syrup is often made by adding sugar to vinegar. Examples of pickling by this method include watermelon rinds (another tradition of the US south), *mostarda di Cremona* (an Italian pickle of mixed fruit and mustard seeds) and pickled walnuts (a British tradition). Such pickles are normally served as accompaniments to savoury meats or cheeses.

Oily Pickles

Oil finds its way into pickles too. Italian antipasti include aubergines and various species of mushroom which are brine-pickled before storage in olive oil. Mustard oil and other vegetable oils are added to dry salted fruit and vegetables (limes, mangoes, tinda, karandas, chillies, aubergines) along with spices to form various Indian pickles.

Pickle Flavourings

In many cases, additional flavouring items are added to the brine or vinegar medium to enhance the general tastiness of the pickle. For example, British pickled onions (but not the silverskin variety, for some reason) are commonly flavoured with various combinations of coriander seeds, cinnamon, bay, cloves and mace (pre-spiced vinegar can be bought for home pickling). Dill is the norm in European and American pickled cucumbers (they are often known as 'dill pickles') and any number of things are added to olives (lemons, oranges, coriander, chilli, garlic, anchovies, wine).

Market potential

Demand and Supply:

Pickles have become an important ingredient of the Indian diet and they are prepared from many fruits and vegetables across the country. They are table enriches and are consumed along with main course as well as many snacks. Pickle made from fish or prawn would also fall in the same category. But its consumption would be limited only to non-vegetarians.

Marketing Strategy

There are very few national brands and bulk of the market is controlled by the local manufacturers. Demand for ready to serve fish products is increasing in the North-East. Apart from individual households, hostels, restaurants, roadside eateries, canteens etc. are bulk consumers.

Proper placement of products, attractive point of sale publicity and handsome commission to retailers shall be the critical aspects.

Process

Raw Materials:

There are six basic types of ingredients used for pickle making. The main bulk food is the cucumber. The additional ingredients include acids, flavorings, colorants, preservatives, and stabilizers that make up the liquid, or liquor, in which the pickle is sold. Many of the ingredients are only available at certain times of the year, so steps have to be taken to use fresh materials.

Undoubtedly, the most important ingredient in pickle manufacturing is the cucumber. Special seeds are used to produce cucumbers that are straight, thin skinned, have a predictable number of warts, and are properly sized. These characteristics are important for uniform pickle manufacturing. Technically, pickles can actually be made using all kinds of foods such as onions, peppers, olives, pears, peaches, and even fish and meat. These are usually referred to as pickled foods to indicate the type of processing required to make them.

Acetic acid (vinegar) is the primary ingredient used in pickle manufacturing. After water, it makes up the bulk of the pickle liquor and contributes significantly to the flavour of the pickle giving it a sour taste. Additionally, it also has a preservative effect and is nontoxic. Vinegar is derived from naturally occurring sugars or starches through a two-step fermentation process. Starch is converted to sugar, which is then yeast fermented to form alcohol. The alcohol is exposed to an acetobacteria, which converts it to vinegar. Vinegar can be obtained from many sources and each one has a slightly different taste. Therefore, depending on its source, the vinegar can have a significant effect on the taste of the final pickle product.

Other ingredients, which impact the final taste of the pickles, are added to the liquor. Sugar is used to provide a sweetness to offset the sour taste of the vinegar. It also helps to make pickles more plump and firm. Artificial sweeteners like aspartame and saccharine can be used for a similar effect without increasing the calories. Salt is added for flavour and it also has an added preservative effect. Pure granulated salt is typically used since it is devoid of anti-caking ingredients that could make the liquor cloudy.

Organic acids and salt(sodium chloride) are the primary preservative for most type of pickles.Acetic acid (or vinegar) is the usual acid added to pasteurized, unfermented (fresh-pack) pickles. Vinegar also is added to many products made from fermented (salt stock) cucumbers. Other preservatives such as sodium benzoate, potassium sorbate, and sulphur

dioxide may be added to finished products. Under Rule of the PFA Rules 1955, preservative have been classified into class I and class II preservative. Class I preservatives belongs to following four distanct taste groups.

i. Saltish- common salt
ii. Sweet- sucrose, dextrose, glucose and honey.
iii. Pungent- spices , vinegar (natural as well as synthetic)
iv. Oily- only edible vegetable oils.Other fats like milk fat, animal fat oils have not been suited.

Class II preservative are,

i. Benzoic acid including salts thereof,
ii. Sulphurous acid including salts thereof,
iii. Nitrates/nitrite of Na/K,
iv. Sorbic acid including its Na/K/Ca salts,

Dill weed is the most common type of aromatic spice and is used to make all forms of dill pickles. Other aromatic spices include allspice, cassia, cinnamon, cloves, fennel, fenugreek, and nutmeg. For more potent pickles, hot spices such as capsicum, black pepper, ginger, and mustard are used. Herbs like basil, marjoram, mint tarragon, and thyme are also used to give pickles a unique taste. Flavourful vegetables including onions and garlic are often included in a pickle liquor. Typically, the pickle manufacturer has a standard spice mix made for each type of pickle they manufacture.

Some additional ingredients may be added to ensure the pickles meet standards set by the manufacturer. In general, pickles do not require any colorants because their natural color is acceptable. However, to create a standardized product and overcome the effects of processes such as bleaching, manufacturers often add color. Two common types of colorants are turmeric caramel and chlorophyll. The caramel provides a slightly brown to yellow color and chlorophyll gives a green color. To inhibit color changes in pickles, sulfur dioxide is added. Firming agents such as lime and alum may also be added. These materials help make pickles crispier without significantly impacting the flavour. Surfactants such as polysorbate are also used to couple ingredients in the liquor solution.

Ingredients:

Fresh peeled prawns	1000 g
Garlic	100 g
Ginger	100 g
Green chillies	80 g
Chilli powder	80 g
Cumin seeds	25 g
Mustard	25 g
Asafoetida	15 g
Fenugreek	10 g
Garammasala	10 g
Turmeric powder	10 g
Curry leaves	5 g
Vinegar	300 ml
Salt	80 g
Citric acid	5 g
Sodium benzoate	0.5 g
Refined sunflower oil	400 ml

Preparation of prawn pickle

Fresh prawns are peeled and washed and then blanched in 6% salt solution containing 0.02% O citric acid at 100^0C for around 15 minutes and then drained and cooled. Cleaned fresh garlic and green chillies are cut longitudinally whereas fresh lemons are cut into 8-10 pieces. Asafoetida is ground and mixed with water or vinegar. Then edible oil is heated to around 200^0C and mustard is fried into it. Subsequently, garlic, green chillies and lemon pieces are mixed with oil one after another and are stirred. Then salt, ginger pieces and asafoetida Solution is mixed. Finally vinegar is added and when there is a separation of liquid portion, blanched prawns are added slowly and mixed well. After heating the mixture at 100^0C for about 3 minutes, it is cooled down to around 60^0C and then preservatives are mixed. On cooling, prawn pickle is packed in air tight bottles.

Peeled prawns and deheaded
↓
Add little salt, turmeric powder and Washing
↓
Fry prawns in hot oil up to they attain golden brown colour
↓
Mince the garlic, ginger, green chilli, in to paste
↓
Fry and powder the fenugreek, cumin seeds, mustard,
and asafoetida.
↓
Mixing
↓
Addition of curry leaves and little sugar in hot oil
↓
Addition of all pastes and fallowed by the powders
and fry until the odour comes.
↓
Add vinegar and salt
↓
Add turmeric powder, chilli powder and garam masala.
↓
Add citric acid and sodium benzoate
↓
Add fried meat to gravy and mix properly
↓
Cooling
↓
Bottles and cleaned and sanitised
↓
Packaging and storing (at room temp).

Flow chart for Preparation of prawn pickle

Preparation of fish pickle: -

In case of fish pickle also fish is cleaned in water and then cleaned pieces are mixed with salt, chilli powder and turmeric for about 2 Hours. Then pieces are fried in edible oil till they are brown in colour. Likewise, onion, garlic and ginger is ground in paste form and then fried till it becomes light brown. Then vinegar is added along with salt, chilly and turmeric powder to the mixture and it is heated till vinegar is absorbed in fish. Then it is cooled and ground spices and preservative are mixed and fish pickle is packed in air-tight bottles. Weight-loss on cleaning etc. shall be 50-55% which will be, to some extent, compensated by addition of other ingredients. Hence, net yield would be 65%.

Ingredients	
Fresh fish meat	1000 g
Garlic	120 g
Ginger	120 g
Green chillies	80 g
Chilli powder	80 g
Cumin seeds	25 g
Mustard	25 g
Asafoetida	15 g
Fenugreek	10 g
Garam masala	10 g
Turmeric powder	10 g
Curry leaves	10 g
Vinegar	300 ml
Salt	80 g
Citric acid	5 g
Sodium benzoate	0.5 g
Refined sunflower oil	400 ml

Raw material.
↓
Washing.
↓
Removal of fillets and de-skinning.
↓
Fillets cut into small cubes.
↓
Fry the cubes in 50% of oil.
↓
Mince the garlic, ginger, green chilli, in to paste.
↓
Fry and powder the fenugreek, cumin seeds, mustard, and asafoetida.
↓
Mixing
↓
Addition of all pastes and fallowed by the powders and fry until the odour comes.
↓
Add vinegar and salt
↓
Add turmeric powder, chilli powder and garam masala.
↓
Add citric acid and sodium benzoate.
↓
Add fried meat to gravy and mix properly.
↓
Cooling
↓
Bottles and cleaned and sanitised.
↓
Packaging and storing.

Flow chart for the preparation of fish pickle

Preparation time: 1 to 2 hrs

Cooking time : 10 to 30 min

Packaging:

After the pickles have adequately fermented, the pickles are then filled without air gaps into glass jar/bottles which are sterilised in an autoclave for 10-15 mins, and then dried. After filling, close the mouth of jar with lids tightly. Before closing clean the lids with vinegar.

Storage

If you follow this basic recipe, you should produce a safe, good quality product. However, the fish must be stored under refrigeration (38˚F) to provide an added measure of safety. This will ensure that food poisoning bacteria will not grow. Refrigerated storage also will retard bacterial spoilage, reduce problems with enzymatic softening, and reduce discoloration. If refrigeration facilities are limited, do not pickle more fish than you can consume.

Nutritional Benefits

According to the NSDA, pickles contain moisture, energy, protein, fat, carbohydrates, dietary fiber and sugars. The minerals found in this pickle include iron, magnesium, phosphorous, potassium, and sodium. This pickle also contains vitamins that include vitamin C, thiamin, riboflavin, niacin, vitamin B-6, folate, vitamin B-12, vitamin A, vitamin E, vitamin D and vitamin K.

Health Benefits of Pickles

Free radical scavenging from the antioxidants

Pickles can be good source of antioxidants. Antioxidants are those micronutrients which help in protecting our body against the attack of free radicals. Free radicals are unstable chemicals which are produced during metabolism. These unstable chemicals react with our cells and damage our DNA to become stable and in the process create more and more free radicals. We can protect ourselves from free radical attack by consuming food with high antioxidants. A lot of emphasis these days is put on antioxidants by dieticians and doctors.

Supply of probiotic or gut-friendly bacteria

Probiotic bacteria are those friendly bacteria which are present in our digestive system. These bacteria actually help us in the digestion of food. Sometimes due to the use of antibiotics, along with invading bacteria, these friendly bacteria are also killed. The fall in their numbers can cause digestive problems which can be solved by eating pickles made without the use of vinegar. Naturally fermented salt pickles encourage the growth of these friendly bacteria which will replenish the numbers in our digestive system and restore our health.

Supply of essential minerals and vitamins

Fresh pickles are interesting and appetizing ways of making children eat their share which otherwise are boring for children. Eating freshly made pickles not only taste good but they also supply essential vitamins such as vitamin C, A, K folate and minerals like iron, calcium and potassium. Vitamins and minerals are vital micronutrients which protect us from diseases, help build immunity, bone strengthening, vision protection, curing anemia and various other functions.

Diabetes control

Studies have shown that taking vinegar based pickles improves haemoglobin levels in diabetic patients, which in turn helps in controlling diabetes. The acetic acid present in vinegar has been noted to be responsible for this phenomenon. But, care must be taken to avoid the consumption of salted pickles as excess salt increases blood pressure.

Liver protection

Apart from benefits such as improved digestion, pickle also has hepatoprotective properties. Studies have shown that when amla extracts were administered on lab animals with chemical induced hepatotoxicity or liver damage, the damage was reduced significantly.

Reduces ulcers

Ulcers are internal wounds caused due to failure of mucus membranes and acid interaction on tissues. Particularly, gastric ulcers are caused by weakening of mucous membrane and due to hyperacidity. Consumption of amla based regularly also helps in reducing ulcers, if any.

Care to be taken while eating pickles

Almost all the pickles contain high amount of salt in them. Salt is an indispensible ingredient in most pickles. It not only adds to the taste, but it helps in preserving the pickle and acts as an anti-microbial, keeping away unwanted bacteria, yeast and fungi. But, excess salt consumption through pickles can bring in problems of its own.

Hypertension is one of the major risks of eating excess salt. Hypertension is known to be one of the reasons causing stroke and heart attacks, especially in older people. Also hypertension increases with increasing age.Apart from the risk of hypertension, Indian pickles may contain high quantities of oil which increases the risk of fat and cholesterol development in our body. Hence, regular intake of pickles must be reduced and must be limited to occasional consumption.

PFA Specification for pickles

Pickle means the preparation made from sound, clean, raw or sufficiently mature fruits or vegetables or a combination of both free from insect damage of fungus attack preserved in salt acid, sugar or any combination of the three. The pickle may contain onion, garlic, ginger, sugar, jaggery, edible oils, spices, spice extract or oil of turmeric, pepper, chillies, fenugreek , mustardseed or powder, vegetable ingredients, asafoetida, bengal gram, lime juice, lemon juice, green chillies, vinegar or acetic acid, dry fruit including raisins and fruits nuts. Pickles shall be free from added synthetic Food colours.

Combination of pickles may be:

(i)Pickles in citrus juice or brine:

The percentage of salt in covering liquid shall not be less than 10 per cent when salt is used as major preserving agent. When packed in citrus juice, acidity of the covering liquid shall be not less than 1.2 per cent calculated as citric acid. Soluble calcium salt and permitted preservatives may be used in soluble calcium salt and permitted preservatives may be used in such type of pickles. Pickles shall be free from copper and alum .

(ii)Pickles in oil.

The fruit or vegetable percentage in the final product shall not be less than 60 per cent. The pickle shall be covered with oil so as to form a layer of not less than 0.5 cm above the contents or the percentage of oil in pickle shall be not less than 10 percent.Pickle shall be free from copper , alum and mineral acid. It may contain rapeseed (rai) ajwain,saunf,black pepper and like spices, etc. Permitted preservatives may be used in Pickles.

(iii)Pickles in vinegar:

Pickles in vinegar mean the preparation from sound, clean, raw or sufficiently matured fruits or vegetables free from insect damage or fungus attack, which have been cured in brine or dry salt or salted and dried stock with or without natural fermentation. It shall contain vinegar or acetic acid and the percentage of acid in the fluid portion shall not be less than 2 per cent w/w calculated as acetic acid. It may contain sugar, whole or ground or semi-ground spices, dried fruits, green and red chillies, ginger, etc. dry fruit. Citric acid may also be added in such type of pickles. Spice extract or essences may also be used. The drained weight of the product shall not be less than 60 per cent. Pickles shall be free from copper, mineral acid, alum synthetic colours and shall show no sign of fermentation. The product shall be reasonably free from sediments. Permitted preservativesmay be used in pickles.

CONCLUSION

According to the latest data on child under nutrition, from 2005–2010, India ranked second to last on child underweight out of 129 countries— below Ethiopia, Niger, Nepal, and Bangladesh. Developing countries account for 98% of the world's undernourished people. Together, China and India alone account for over 42 percent (231 million in India and 123 million in China) of the chronically hungry people in the developing world. The Food and Agriculture Organization of the United Nations (FAO) estimates that 22% of India's population is undernourished. So by maximising the use of fishery based products this problem can be solved. Prawn and fish pickle is one of the most important value added product,it can supply animal protein to humans which is most preferred one & also helps in reduction of protein based malnutrition by providing nutrient security directly & food security, social development & economic development indirectly. Pickle are being said a good source of anti-oxidents will help in protecting body against the attack of free redical. Due to addition of preservatives shelf life is increased for several months & small size prawns and low value fishes can be utilized for also for making pickle.

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